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FACTORS AFFECTING THE DEVELOPMENT OF RHYNCHOSPORIUM SCALD ON BROME GRASS[1]

Edgar V. Christensen and Roy D. Wilcoxson[2]

SUMMARY

Temperatures near 18°C were most favorable for infection of brome grass *(Bromus inermis)* by *Rhynchosporium secalis* in moist chambers. Concentration of inoculum and time in moist chambers acted independently and together to control the severity of infection. When all other conditions were favorable for infection, removing inoculated plants from moist chambers and drying their surfaces rapidly at 29°C reduced infection. More infection centers formed on leaves rubbed before inoculation than on those not rubbed, and young leaves were more susceptible than older ones, presumably because of protection offered by the cuticle. Clones resistant or susceptible in the greenhouse had similar reactions in the field.

Scald caused by *Rhynchosporium secalis* (Oud.) J. J. Davis is one of the common diseases of *Bromus inermis* Leyss., and damage is sometimes serious. Since most of the work with Rhynchosporium scald has been done with barley (1, 3, 4, 5, 6, 8), the present work was undertaken to study factors affecting the disease on brome grass.

METHODS AND RESULTS.—Clonal lines 79-2 and 99-4[3] of brome grass, both susceptible in the field, were removed from the field, divided into portions, and subsequently propagated in 4-in. pots in a greenhouse at about 22°C to ensure genetic uniformity. Only plants of uniform size and age (1 shoot per pot) were used in the experiments.

Cultures of *R. secalis* (7) were obtained from brome grass collected in Minnesota, Wisconsin, and Pennsylvania in 1956 and 1957. Stock cultures were maintained on Lima-bean agar and stored at 20°C. Inoculum was produced on an autoclaved 3:1 mixture of barley and wheat grain in the laboratory at temperatures fluctuating between 18° and 29°C. Conidial and mycelial fragments were decanted from the grain mixture in either tap or distilled water, and conidial concentrations were determined with a Brightline haemocytometer.

At inoculation the leaves were counted and the inoculum plus Polyethylene glycol 400 (monolaurate) (1 drop in 50 ml of inoculum) was sprayed onto the plants for 15–20 seconds at about 3 lb. pressure. As the spray was applied the plants were rotated slowly on a turntable.

Inoculated plants were kept moist in sheet-metal boxes standing in trays of water with temperatures varying from 18° to 24.°C. The moist chambers were covered with polyethylene sheets, which permitted some light of very low intensity to enter. As moist-chamber treatment ended, plants were removed to greenhouses maintained at about 18°, 22°, 24°, and 29°C, where they were either held until notes were taken or subjected to further treatment.

Growing and storing inoculum.—Growing and storing *R. secalis* on a mixture of barley and wheat proved a satisfactory technique for use in greenhouse and field work. On agar media the fungus grew slowly but on grain medium it grew vigorously and formed abundant conidia within 2 weeks. At intervals of 11, 18, 42, 70, and 77 days after the grain was seeded with the fungus, the viability and virulence of the fungus was tested, and the data indicate that inocula of these various ages are equally effective.

Moist-chamber treatments.—Eighteen pots of clone 79-2 were sprayed 20 seconds with a suspension containing 400,000 conidia per ml, held 48 hours in moist chambers under shade in greenhouses at 18°, 24°, and 29°C, and then kept in a greenhouse at 22°C until notes were taken. The experiment was repeated 3 times. The average number of lesions per leaf decreased as the temperature increased. The average number of lesions at 18°C was 15.8, at 24°C it was 10.4, and at 29°C it was only 3.0. The decrease in lesion number between 18° and 29°C was statistically significant (HSD[4] at 5% level = 10.89).

Conidial concentration and duration of the moist period.—Plants of clone 79-2 were inoculated (15-second application) and held in moist chambers according to the schedule in Table 1. An orthogonal rotatable experimental design was used.

The data indicate that both inoculum concentration and time in moist chambers alter the amount of infection, and, according to the statistical analysis, the linear regression was significant at the 1% level. The effect of inoculum concentration was illustrated when suspensions containing 50,000 and 500,000 conidia per ml on plants in moist chambers 54 hours resulted

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[3] Pedigrees of these clones are located in the field book pertaining to Ives' M.S. thesis, Department of Agronomy and Plant Genetics, University of Minnesota (2).

[4] HSD = honest significant difference (9).

TABLE 1.—*Lesions produced by Rhynchosporium secalis on brome grass with varied inoculum concentration and time in moist chambers*

Inoculum concentration (conidia/ml)	Hours in moist chamber	Lesions per leaf [a]
135,000	24	0.6
400,000	24	2.0
275,000	46	5.4
50,000	54	1.5
500,000	54	14.0
275,000	72	10.9

[a] Averages of 12 plants in 2 replications. The linear regression was significant at the 1% level.

in 1.5 and 14 lesions per leaf, respectively. Differences because of the duration of the moist period were shown when suspensions containing 275,000 conidia per ml on plants in moist chambers 46 and 72 hours resulted in 5.4 and 10.9 lesions per leaf, respectively. The interaction of the 2 variables was indicated when about 2 lesions per leaf formed on plants inoculated with suspensions containing 50,000 and 400,000 conidia per ml and held in moist chambers 54 and 24 hours, respectively. The combination of a suspension containing 135,000 conidia per ml and 24 hours in moist chambers resulted in an average of less than 1 lesion per leaf.

Rapid and slow drying treatments.—Clone 79-2 was sprayed 20 seconds with a suspension of 500,000 conidia per ml, and held 54 hours in moist chambers, and a third of the plants were then taken into each of 3 greenhouses at 18°, 24°, and 29°C. There, half of the plants were placed on the bench and the other half were put in chambers covered with 3 layers of cheesecloth. Plants on the benches were apparently dry within 5 hours (rapid drying), whereas those in the chambers did not become dry until after 10 hours (slow drying). After being in these conditions overnight the plants were removed to the 22°C greenhouse and held until lesions appeared. The experiment was repeated 5 times and the data are presented in Table 2.

Rapid drying at 29°C reduced the average number of lesions per leaf from 12.3 to 7.3, a statistically significant difference. Slow drying at all 3 temperatures resulted in an average of 11 lesions per leaf, whereas rapid drying resulted in 9.6 lesions per leaf. This difference was almost statistically significant at the 5% level. Differences in the amount of infection due to temperatures at which plants were dried were not statistically significant.

The effect of cuticle on infection.—Leaves of clone 99-4 were rubbed between the fingers encased in heavy canvas gloves, and these plants and nonrubbed checks were then sprayed 15 seconds with a suspension containing 1 million conidia per ml, held 48 hours in moist chambers, and placed in the 22°C greenhouse. The experiment was repeated 3 times.

In 2 of the trials an average of 11.4 lesions per leaf formed on rubbed plants whereas an average of 2.3 lesions per leaf formed on nonrubbed plants, a statistically significant difference. In the third trial a similar difference in the amount of infection on rubbed and nonrubbed leaves occurred, but the difference was not statistically significant. Plants used were younger and more succulent in trial III than in the other 2 trials, which may account for the results.

If the cuticle on nonrubbed leaves hinders infection, it is probable that the number of lesions will be greater on younger leaves than on older leaves. In 11 experiments involving 5808 leaves, the number of lesions was recorded on leaves of different ages on the same plant. As indicated in Fig. 1, there were more lesions on younger leaves than on the older leaves (correlation coefficient $r = 0.856$). The variations in the slope of the curve at leaves 3 and 8 were not statistically significant.

Clonal resistance.—Nine clones were established in a field nursery in 1957. In April, 1958, the plant debris was burned with a flame thrower, which, along with unusually dry weather, resulted in disease-free plants. On May 30, 1958, a rainy weekend was forecast, and the plants were sprayed with a suspension containing about 1 million conidia per ml, and grain that carried inoculum was scattered among the plants. The nursery remained wet for about 36 hours after inoculation. Disease notes were taken 76 days later, on August 14, 1958, by scoring each plant for severity of infection on the following scale: 1 = none, 2 = light, 3 = moderate, and 4 = heavy.

The average disease rating for clones 95-2, 99-4, 86-2, and 79-2 varied from 3.3 to 4.0, and the average disease rating for clones 2-1, B 38, 4-5, 148-3, and B 17 varied from 1.0 to 1.5. The difference between the resistant and the susceptible clones was statistically significant but differences between replications were not. In the greenhouse, clones 95-2 and 99-4 were rated susceptible, and 148-3 was rated resistant. This agrees with the field evaluation and suggests that preliminary tests in the greenhouse would be useful for selecting resistant plants.

DISCUSSION.—Rhynchosporium scald appears in the field during cool seasons, and is usually more common in the spring than the fall. This seasonal cycle has been attributed to the inhibitive action of high temperatures (1, 4), which is supported by the observation that more lesions formed on plants held in moist chambers at 18°C than on plants held at higher temperatures.

In addition to temperature, 3 other environmental

TABLE 2.—*Lesions produced by Rhynchosporium secalis after brome grass plants were dried slowly or rapidly at 3 temperatures*

Rate of drying	Av. no. lesions per leaf 18°C	24°C	29°C	Av. for rate of drying
Rapid [a]	9.1 [b]	12.6	7.3	9.6
Slow [c]	10.0	10.7	12.3	11.0
Average for temperature	9.5	11.6	9.8	

[a] Dry within 5 hours.
[b] Average of 30 plants in 5 replications. Rate of drying and temperature interaction HSD 5% = 4.38.
[c] Dry after 10 hours.

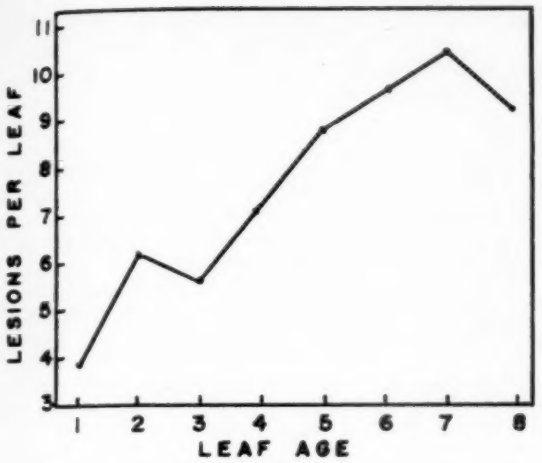

Fig. 1. Leaf age and number of lesions formed by *Rhynchosporium secalis* on brome grass. Leaf 1 is the oldest. The correlation coefficient r is 0.856.

factors also restricted the development of infection, and these factors are probably also more favorable for the disease during cool seasons. The concentration of inoculum and the time in moist chambers were important, singly and in combination, in altering the amount of infection. The data indicate that about equal levels of infection can be obtained when smaller doses of inoculum are combined with longer times in moist chambers, and vice versa. The third factor was the speed and temperature at which plant surfaces dried off after other provisions had been made to ensure abundant infection. Thus, (at 29°C) plants that dried off rapidly had about half as many lesions per leaf as did plants that dried off slowly. Speed of drying at 24° and 18°C did not appear to alter the number of lesions significantly.

The nature of resistance has not been studied extensively. In experiments reported herein, infection was about 5 times as great on leaves rubbed before inoculation as on nonrubbed leaves, and more infection developed on younger leaves than on older leaves. This suggests that cuticle is important in the resistance of brome grass to attack by *R. secalis*.

Since the disease reactions of 3 clones were the same in both greenhouse and field tests, preliminary trials for resistance would be feasible in the greenhouse in eliminating susceptible lines of grass from breeding programs.

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MECHANISMS OF INFECTION OF SWEET POTATO ROOTS BY RHIZOPUS STOLONIFER[1]

D. N. Srivastava[2] and J. C. Walker

SUMMARY

Germ tubes arising directly from the spores of *Rhizopus stolonifer* were nonpathogenic on wounded sweet potatoes, but, when allowed to grow on nutrients for 24–48 hours, produced infective hyphae that readily incited the disease. Failure of the spores to infect through sharply cut wounds was not due to wound cork formation, since inoculation through such wounds with hyphae from a 2-day PDA (potato-dextrose agar) culture of the fungus gave consistent infection. Cultures lost pathogenicity with age. Optimum temperature for development of the disease in sweet potato was 20°C, and optimum for growth on PDA was 28°. The discrepancy between the 2 optima is explained by the fact that pectolytic enzyme production by the fungus on sweet potato substrate is greatest at 20° and thus is not correlated with growth on PDA.

Once infection was established, the disease progressed normally at a relative humidity range of 50–100%. In nature the initiation of decay is most common at the spindle ends. Spores proved capable of initiating infection at the spindle ends when dead tissue supported the production of infective hyphae.

In a study of the nature of exoenzymes secreted by *Rhizopus stolonifer* (Fr.) Lind. (11) it became desirable to use sweet potato roots as a natural substrate. It was found, as reported by Harter, Weimer, and Adams (6), from an extensive study of this disease, that, although the pathogen penetrates primarily if not entirely through wounds, artificial infection through wounds was seldom attained in high percentages unless special methods were employed. They developed such a method, which they called the "well method." Lauritzen and Harter (8) and Weimer and Harter (14) also noted that the optimum temperature for growth of the organism in culture was distinctly higher than that for development of the disease in sweet potato. The present investigation is a study of the mechanisms of infection of sweet potato roots, and the relation of temperature, humidity, and aeration to disease development.

METHODS AND MATERIALS.—A culture of *Rhizopus stolonifer*, obtained from K. B. Raper, was labeled strain NRRL 1477 (+). When the culture was mated with a (−) strain, secured from M. P. Backus, typical zygotes were formed. The (+) strain was used throughout this investigation. The sweet potato roots used, from the Madison (Wisconsin) market, were mostly the variety Porto Rico. Before inoculation they were washed with water and dipped in 95% alcohol. Ordinary glass desiccators about 12 in. in diameter were used as moist chambers for incubating inoculated material. Moist paper towels in the chambers raised the relative humidity, which was adjusted to the desired approximate level by leaving the lids open by appropriate amounts. Relative humidity was determined by placing a Type HGS-HY-1 hygrometer inside the moist chambers for 15–20 minutes.

EXPERIMENTAL RESULTS.—*Reproduction of the disease under laboratory conditions.*—In contrast to the widespread occurrence in nature of the *Rhizopus* soft rot of sweet potato, efforts to reproduce it artificially under laboratory conditions are often unsuccessful. In the "well method" (6), the fungus from a 48-hour sweet-potato-broth culture is placed, together with a small quantity of the broth, in a well made in the potato. To prevent evaporation, the well is then sealed with a cover slip by means of Vaseline. Since this method is rather cumbersome, a search was made for a better one.

Ten healthy sweet potatoes were each inoculated through a circular wound made by removing with a knife a piece of tissue about 2 cm in diameter and 1 cm deep midway between the 2 ends of the spindle. The inoculum consisted of a small quantity of aerial mycelium and spores transferred to the wounds from a 10-day PDA (potato-dextrose-agar) culture grown at 27°C. The tissue removed was then gently placed on the inoculum. The inoculated potatoes were kept in a moist chamber at 27°C and 95–100% RH (relative humidity). Observations made after 10 days showed that none of the potatoes had rotted. Lowering the temperature and changing the humidity level did not alter the results. When, however, the inoculum consisted of aerial hyphae and spores taken from a 4-day culture grown at 27°, 5 of 20 potatoes rotted in 3 days. This result led to a study of the relationship of age of the culture to its pathogenicity.

Healthy sweet potatoes were inoculated by the same method with cultures grown on PDA at 20°C for 2, 4, 6, 8, 10, and 15 days. The inoculum consisted of a 1-cm^2 agar block supporting mycelium and spores, except that the 2-day culture had not formed spores. The inoculated potatoes were incubated in moist chambers at 20° and 95–100% RH. As shown in Table 1, a 2-day PDA culture, consisting only of actively growing hyphae, was capable of producing 100% infection. As the cultures aged and mature conidiophores and conidia were produced, there was a progressive decline in pathogenicity up to 15 days, at which time the fungus was nonpathogenic. The

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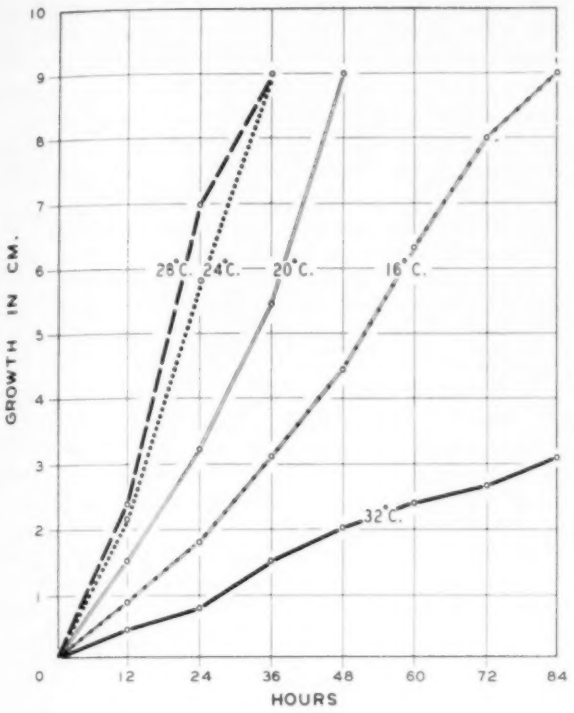

Fig. 1. Rate of growth of *Rhizopus stolonifer* on potato-dextrose agar at different temperatures.

pathogenicity of the culture, therefore, depended on the actively growing hyphae, whereas spores and old hyphae appeared to be noninfective. Other experiments showed that the culture lost its pathogenicity more rapidly at a room temperature of 27° than at 20°.

In further experiments it was found that the agar block was not necessary in transferring inoculum to the wound. If the young, undifferentiated erect hyphae were gently lifted by a needle and transferred to the wounds, they brought about 100% infection, although the start of rotting was slightly delayed. Thus, a method was developed by which the disease could be reproduced with certainty. The 2-day inoculum used in this method is herein referred to as "standard inoculum," and the method as the "plug" method. Harter, Weimer, and Adams (6) reported failure to reproduce the disease by using the rotted sweet potato tissue as the inoculum. In the present study, inoculations brought about infection as readily with rotted tissue as with standard inoculum.

TABLE 1.—*Effect of age of the culture on pathogenicity of Rhizopus stolonifer on sweet potato*

Age of culture (days)	Potatoes inoculated (no.)	Per cent of potatoes rotted in 8 days
2	10	100
4	10	90
6	10	90
8	10	70
10	10	60
15	10	0

The above results indicate that spores applied directly to wounds by the plug method do not cause infection. Before proceeding further to investigate the relation of spores to initiation of disease it was considered desirable to study the relationship of environmental factors to infection and disease development.

Relation of temperature and humidity to fungus growth, infection, and disease development.—The rates of growth of the fungus were determined at 16, 20, 24, 28, and 32°C. Five Petri dishes 9 cm in diameter were inoculated for incubation at each temperature. Inoculum was a small quantity of agar, about pin-head size, supporting actively growing mycelium. Radial growth measurements were taken at 12-hour intervals in each dish, along 2 diameters of the colony at right angles to each other. The average of the 10 readings was determined for each temperature. Growth was most rapid at 28° (Fig. 1).

Ten medium-sized sweet potatoes were inoculated by the plug method with standard inoculum. The inoculated potatoes were incubated in moist chambers at 16, 20, 24, 28, and 32°C. RH in each case was maintained at 95–100%. After 3½ days, the rotted potatoes were split open longitudinally and the linear spread of the decay was recorded. The experiment was repeated, and the combined data are presented in Fig. 2. Decay

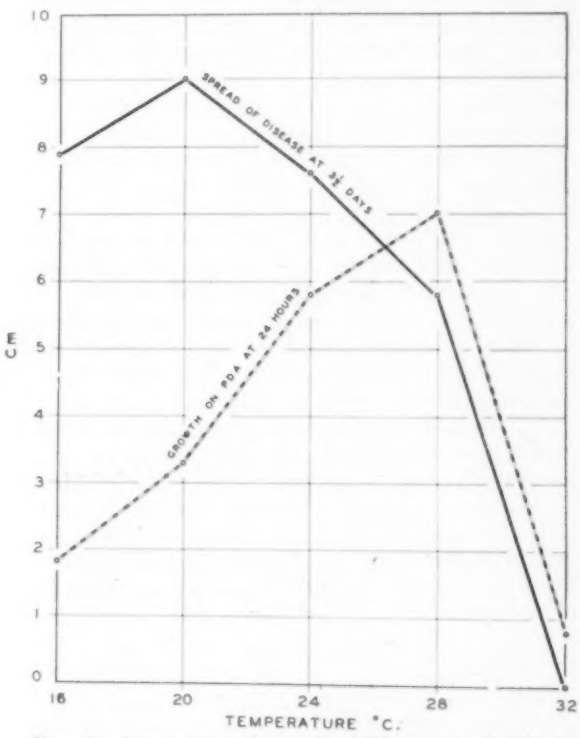

Fig. 2. Comparison of growth of *Rhizopus stolonifer* on potato-dextrose agar and disease development in sweet potato roots at different temperatures.

TABLE 2.—*The relation of temperature to the enzymic activity of Rhizopus stolonifer on sweet potato tissue as measured by maceration time, PME activity, and PG activity of rotted tissue extracts*

Temperature at which potatoes rotted (°C)	Activity of extracts		
	Maceration time (minutes)	PME activity[a]	PG activity[b]
16	48	3.3	0.2
20	40	6.2	0.3
24	48	5.6	0.1
28	60	5.3	0.1

[a] Expressed in mg methoxyl groups removed by 1 ml of extract at 3 hours.
[b] Expressed in ml of 0.1*N* iodine solution reduced by 5 ml of reaction mixture.

at 3½ days was most extensive at 20°C. It was slightly less at 16° and 24° than at 20°, still less at 28°, and absent at 32°. An optimum temperature of about 20° for development of the disease was also reported by Lauritzen and Harter (8). Optimum temperature for the growth of the fungus on PDA is seen to be 28°, as against the optimum of 20° for disease development.

As shown in a previous paper (11), the fungus excretes pectolytic enzymes and a cellulase (Cx) to cause the macerating action that leads to decay of sweet potato tissue. To determine enzyme production rates at different temperatures, sweet potatoes were inoculated with standard inoculum by the plug method, and placed in moist chambers at 95–100% RH and at temperatures of 16°, 20°, 24°, and 28°C, respectively. After 6 days the crude juice was extracted from each lot of roots and centrifuged. The enzyme activity of the extracts was tested in 3 ways. The gross macerating action was determined by placing carrot-root sections in dishes containing the extract diluted 1:3 with water and determining the time required for maceration to proceed to the point when the sections could not be raised with a forceps. The PME (pectin methyl esterase) activity and the PG (polygalacturonase) activity of each extract were determined by the methods described by Winstead and Walker (15). The results are in Table 2. It is obvious that macerating action, PME activity, and PG activity were all highest at 20°, which coincides with the temperature at which the potatoes rotted most rapidly. It appears that on PDA, where the fungus has a direct supply of nutrients in simple form and does not need pectolytic action on the substrate to provide available carbohydrates, the rate of growth is determined by other metabolic activities, with an optimum around 28°. On sweet potato substrate, however, the secretion and activity of extracellular enzymes and the consequent breakdown of middle lamellae are apparently essential to provide available carbon sources through hydrolysis of carbohydrates and the stimulation of exosmosis. This is the most plausible explanation of the fact that the optimum for disease production is several degrees lower than that for growth on PDA.

Brooks and Cooley (1) reported than the optimum temperature for infection and disease development in peach rot incited by *R. stolonifer* is around 30°C, 2° above the optimum for its growth on PDA. It may be pointed out that, in nature, this fungus attacks only peaches that are ripe. The substrate of ripe peaches, unlike that of sweet potatoes, may be richer in nutrients immediately available to the fungus without the action of pectolytic enzymes. This may account for the fact that the optimum temperature for peach rot is in the vicinity of the optimum for growth on PDA. It would, however, be interesting to determine whether unripe peaches could be infected by spores or the standard inoculum and, if so, where the optimum temperature might lie.

Medium-sized sweet potatoes inoculated with standard inoculum by the plug method were incubated at 20°C in moist chambers with RH of 95–100%, 75–85%, and 50–60%. Final observations were recorded after 5 days. No appreciable differences were noticed among the 3 levels of humidity in rate of development of the disease. However, moisture in the vicinity of the inoculum had to be sufficient to prevent desiccation of the infective hyphae. This moisture was provided for some time by cell sap present on the freshly cut surfaces of the host as well as by the agar block, even when the RH of the chamber was adjusted to 50–60%. Humidity was thus important in establishing initial infection. Following infection, disease progress was not appreciably influenced by aerial humidity. The results on the moisture relations of the disease are not in accord with those reported by Lauritzen and Harter (9). Using a mixture of spores of *R. stolonifer* and *R. tritici* as inoculum, they reported that an RH of 75–83% was the optimum for infection, with few cases of infection at 93–100% RH. The differences between the results obtained here and those of Lauritzen and Harter might be explained on the basis of the fact that they used spores of 2 species of *Rhizopus* without freeing them from the hyphae.

Effect of aeration on disease development.—In the earlier experiments it was noted that, when the moist chambers in which the inoculated potatoes were incubated were kept airtight, progress of the disease was much slower than when an opening was provided to allow gas exchange. The rapidity with which the disease progresses is bound to result in a rapid uptake of oxygen and release of carbon dioxide. Weimer and Harter (12), using *R. tritici*, found that the inoculated halves of sweet potatoes gave off 6–7 times as much carbon dioxide as the corresponding healthy halves. To study the effect of aeration on development of the disease, an experiment was set up in which 10 sweet potatoes were inoculated with standard inoculum by the plug method and incubated at 20°C in moist chambers at 95–100% RH with the lid tightly closed. In the control the lid of the moist chamber was left slightly open for aeration. The potatoes in the aerated chamber had rotted almost completely at the end of 4 days. Those in the tightly closed chamber were only slightly rotted around the points of inoculation.

In another test inoculated sweet potatoes were kept in airtight as well as open plastic bags at 20°C. Uninoculated controls were also kept in airtight bags. After 15 days, 18 of 20 potatoes in open bags were completely decayed. Of 20 inoculated potatoes in airtight bags, all showed incipient infection but only 3 showed slight rotting; this was about the same as with 20 uninoculated potatoes in airtight bags of which 6 showed incipient infection and 4 a slight rotting.

The fact that lack of aeration checked the progress of the disease can hardly be questioned, but considerable moisture accumulated in the airtight bags to induce partial rotting of apparently healthy potatoes carrying dormant infections at the spindle ends. Besides, storage for 15 days in airtight bags caused abundant growth of other molds on the surface of the potatoes. Control of the disease by packing potatoes in plastic bags is apparently not feasible.

Pathogenicity of spores.—Since it was noticed under laboratory conditions that old mycelium as well as spores did not incite the disease, the noninfectivity of the spores was studied next. Spores free of hyphae were obtained by filtering suspensions of spores and mycelium through ordinary filter paper. Repeated attempts involving inoculation of over 150 sweet potatoes were made in several ways to induce development of the disease by spores. Spores from cultures of different ages, addition of sweet potato juice or extracts of 48-hour mycelium to the inoculum, change of site of inoculation to the tips of the spindle, cooling of the potatoes to 10°C before inoculation, different combinations of temperature and humidity, all failed to induce the disease as long as the inoculum was only spores.

As high as 100% germination was observed in the spores washed from the surface of inoculated wounds after 24 hours of incubation. In certain cases the germ tubes were seen to grow into long hyphal threads. Hyphae characteristic of the fungus were often seen between the cells of apparently healthy tissue immediately beneath the inoculated surface. At times, hyphal strands growing ineffectively on the inoculated wounds could be seen with the naked eye, and a slight localized decay sometimes resulted, but without further progress. To eliminate the possibility that the host developed a mechanical barrier, through suberization or corking beneath the wounded surface between inoculation and the time the spores germinated, the spores were germinated beforehand in the potato juice. Results obtained with such germinated spores were similar to those obtained with ungerminated spores.

Hasselbring and Hawkins (7) showed that the sweet potato has a progressive increase in sugar content with length of storage. The commonly observed increase in percentage of rotting potatoes with time in storage was attributed by them to the increase in sugar content. However, Harter, Weimer, and Adams (6) ruled out such a possibility, for stored sweet potatoes could be rotted by the well method with equal ease at any time of the storage period. They further contended that there is enough sugar in the potato at any time in the storage period to supply the needs of the fungus, and hence the sugar content could not be a factor controlling infection. In the course of the present investigation, potatoes secured from the market near the end of the storage period, in June or July, gave an average of 30% infected roots when inoculated with spores by the plug method. This is in contrast with the rare infection obtained during the early months of storage. Thus, potatoes stored for several months can be infected by spores to a limited extent.

It may be pointed out here that the inoculum employed in the well method by Harter, Weimer, and Adams (6) is similar in effect to the standard inoculum used in this investigation. It is possible that the germinated spore has a higher nutrient requirement to reach a pathogenic stage than is normally available on the wounded surfaces of the potatoes, or that the young sporeling may be competing unsuccessfully at this time with the wounded host tissue for essential metabolites. The higher sugar content of the older potatoes might, therefore, be a contributing factor to establishment of infection by the spores. An experiment was carried out in which 2 ml of 10% glucose solution was poured along with the spores into the wounds of each inoculated potato. Of 20 potatoes so inoculated, none rotted. The failure to secure infection by adding glucose solution does not, however, imply that freshly dug potatoes and those stored 10 months are alike in reaction to the pathogen.

Attempts were also made to incite rotting by spores by adding pectolytic enzymes to the inoculum. Freshly extracted juice from rotted potatoes shows very high activity of the pectolytic enzymes (11). Two ml of this extract was poured along with the spore suspension into the wounds of each potato. Of 20 potatoes so inoculated, none showed rotting during the first 5 days, but 11 were rotted on the 10th day. Appearance of the disease was probably delayed because the spores required time to establish colonies before infective hyphae were produced. Food for this purpose was probably made available from the host tissue by action of the added enzyme.

Harter and Weimer (5) were able to infect potatoes by inoculating spores on wounds made by striking the edge of a wire basket against the potato, or on potato tissues charred by a flame. In this investigation, spores were placed on wounds ¼–½-inch deep made by repeatedly striking the potato with the blunt edge of a knife. Uninoculated controls were also kept. Both inoculated and uninoculated lots were incubated at 20°C and 95–100% RH. Decay developed slightly more slowly than in experiments where standard inoculum was applied by the plug method. Observations were taken at 5 days. Of 20 inoculated potatoes, 17 were decayed; of the uninoculated potatoes, 9 rotted, probably as a result of inoculation by spores in the atmosphere, or on the surface of the potato.

Wound healing in relation to infection.—A widely practiced method of preventing *Rhizopus* soft rot is a curing after harvest by exposing the potatoes for several days to about 30°C and 95–100% RH. The pur-

TABLE 3.—*Effect of healing of wounds at 32°C upon infection of sweet potatoes by Rhizopus stolonifer*

Treatment	Potatoes inoculated (no.)	Potatoes rotted (no.)
Healed 3 days	10	6
Healed 7 days	20	9
Healed for an unknown but longer period	10	0
Unhealed	10	10

pose is to hasten healing of harvest wounds, which may provide avenues of infection. Weimer and Harter (13) have shown that healing is due to the suberization of a few layers of cells beneath the wounded surface, followed by development of a cork layer further below. They found that, under laboratory conditions, cork formation was only partially effective in preventing infection. They could not induce cork formation on wounded potatoes kept under the conditions existing in the storage house but reported the formation of a hard crust that prevented infection.

Using the standard inoculum, a study was made of the effect on infection of the healing of potatoes. Wounds in potatoes healed 3 and 7 days at 32°C and 95–100% RH were inoculated with the standard inoculum. Care was taken to avoid bruising the healed wounds. Potatoes with harvest or storage wounds that had been healed for unknown periods were collected from the market and inoculated in the same way. As controls, potatoes were inoculated immediately after wounding. All potatoes were incubated at 20°C and 95–100% RH. Results are presented in Table 3. It was not determined whether the reduction in percentage of infected roots was due to wound cork formation or to the formation of a latex crust. Inoculated wounds of long standing that had healed in the form of a thick, warty crust were found to be impregnable to the fungus.

Origin and spread of the disease in nature.—When cartons of sweet potatoes in the Madison retail market were examined from time to time, *Rhizopus* soft rot was almost invariably encountered. In one case, 22% of the potatoes in a single box were decayed; only rarely were all potatoes in a carton healthy. Of 100 potatoes showing varying degrees of soft rot that were examined in the market over several months, 96 had symptoms of the disease at the spindle ends, 89 at one end only, and 7 at both ends. In the remaining 4, the disease had originated in wounds at points other than the ends. The consistent origin of infection at the spindle ends was observed 40 years ago (6). Harter and Weimer (5) suggested that protection by wound cork formation and/or by sealing of the wound by latex was less effective in this region than elsewhere on the root.

Since, in nature, the fungus is disseminated as spores, there must be some point on the sweet potato vulnerable to infection by spores to account for the fact that this is the main vegetable host for the parasite (though several other vegetables decay as readily when inoculated with standard inoculum by the plug method). It was found that a certain amount of dead tissue at the tips of the spindle ends of most healthy potatoes was visibly attacked by saprophytic organisms. A study was made of how commonly the *Rhizopus* soft rot organism was present in such dead tissue and how much it could contribute to development of the disease under conditions optimum for rotting.

A carton of sweet potatoes was divided into 2 lots. In one lot, the ends of the potatoes were left intact, and in the other, ½-in. pieces were removed from both ends of each potato. All were incubated at 20°C and 95–100% RH. The removed ends were collected and incubated separately under similar conditions. Observations were recorded at 10 days. The experiment was repeated with a second lot of potatoes, obtained from the same market several weeks later.

The results (Table 4) show that the spindle ends of a large number of potatoes either carried dormant infection or were subject to infection by spores. When such potatoes were subjected for a protracted period to conditions optimum for rotting, the disease developed in many. It is also seen that removing the tips of the spindle largely prevented the disease. The results obtained in experiment 2 show that the carton from which the potatoes were selected consisted of very healthy stock. In fact, the remaining 70 potatoes from that carton were kept at 12°C for several months without a single case of decay.

The next question was how and why the spindle ends become infected. Three lots of healthy sweet potatoes were selected. In the first lot, about 1 cm of the tip of one spindle was removed by a knife, and about 0.5 cm of the tip of the other was crushed with pliers. The second lot was handled similarly except that the tip of one spindle was removed and 1 cm of the tip of the other was crushed. In the third lot both ends were left intact. The ends of each potato were dipped in a suspension of spores, and the 3 lots were incubated separately at 20°C and 95–100% RH. Observations were recorded on the 12th day. It is seen (Table 5) that very little rot occurred in potatoes with sharply cut ends, whereas a high percentage were infected when ends were crushed. The initiation of infection at the spindle ends by spores appears to depend on a certain quantity of dead tissue for production of infective hyphae and subsequent development of the disease. Such infections could take place

TABLE 4.—*Extent of dormant Rhizopus infection at spindle ends of sweet potatoes collected in the Madison market*

Treatment	Exper. no.	No. incubated	% rotted
Spindle ends left intact	1	47	17.0
	2	30	3.3
Spindle ends removed	1	38	2.6
	2	30	0.0
Spindle ends removed from above treatments	1	76	12.0
	2	60	3.3

TABLE 5.—*Development of Rhizopus soft rot in sweet potatoes injured in various ways at the ends of the spindle and dipped in a suspension of spores of Rhizopus stolonifer*

Treatment	No. ends incubated	No. showing visible fungus growth	% incipient rotting	% advanced rotting
Ends crushed less than 0.5 cm	10	10	80	20
Ends crushed to 1 cm	10	10	10	90
Sharply cut ends	20	0	0	5
Ends intact	20	13	40	10

during harvest or at any time afterwards. Death of the tissue at the tips may result from desiccation or the pressure of potatoes in storage. Since the pathogen is nearly ubiquitous in spore form, the inoculum may be expected to be usually present.

DISCUSSION.—It was seen that the germ tubes and the hyphae arising directly from the spores, though nonpathogenic, produced infective hyphae and became pathogenic if allowed to grow for some time on nutrients. The germ tube and the infective hyphae are then physiologically different from each other, the former lacking the substances required to initiate infection and the latter being fully equipped with these mechanisms. The failure of spores to infect sharply cut wounds is attributed to their inability, for lack of nutrients, to grow and produce the infective hyphae. Inability of the spores to infect through sharply cut wounds cannot be attributed to wound cork formation, because infection through such wounds does not take place even if the inoculation is made with spores germinated beforehand.

The striking difference between the germ tube of the spore and the infective hypha in respect to behavior on the host is of fundamental significance to knowledge of the physiology of parasitism. The results obtained here throw further light on the nutrition-drop theory of DeBary (3) on the parasitism of *Sclerotinia sclerotiorum* (Lib.) d By. DeBary stated that host materials that diffused into the infection drop were of nutrient value to the fungus, and that their presence in the infection drop was necessary to convert the organism to one with parasitic activity. In a recent paper McLean (10) showed that the germ tubes of the ascospores of *Sclerotinia sclerotiorum* do not penetrate and bring about infection of cabbage seed plants unless nutrients are furnished to the fungus in the infection drop by petals from mature blossoms that adhere to the foliage. A like situation is seen in the case of *Rhizopus* soft rot of sweet potatoes.

Why the infective hyphae of *R. stolonifer* are capable of parasitic activity is a question that can be answered only by a critical study of the physiology of the fungus. Harter and Weimer (4) reported that pectinase content is maximum in a 2–3-day-old culture of *R. tritici*. A similar situation in *B. cinerea* was reported by Brown (2). If pectolytic enzymes alone are responsible for the parasitism of the fungus, then adding the enzymes of the fungus to the spore suspension should have given rapid infection, but it did not. Further, there are many other fungi that produce pectolytic enzymes in abundance but do not parasitize sweet potatoes. On the other hand it has been shown that *R. stolonifer* is not a specialized pathogen of sweet potatoes, for it can be made to rot almost any other fleshy vegetable but onion. Pathogenicity of the infective hypha may then be due to its ability for continued production of concentrated enzymes capable of rapidly disintegrating the host tissue. It is likely that some other metabolites of the fungus may also be involved in this complex relationship.

It has been demonstrated that the spores, though noninfective through sharply cut wounds, can cause infection through crushed tips of the spindle; and, in nature, a considerable number of apparently healthy potatoes are infected at the ends of the spindle. The production of the infective hyphae from spores and the resulting incipient infection at the tips of the spindle vary with the extent of dead tissue available there. If the dead tissues are adequate, the fungus grows and produces the infective hyphae, but the subsequent development and spread of the disease are determined by the environmental conditions that follow.

The reason why curing is effective in counteracting this disease, then, lies essentially in the elimination or retardation of the incipient infection at the tips rather than in suberization or wound cork formation. At the high temperature, humidity and carbon dioxide concentration in the microclimate of the curing houses, the spores presumably germinate, and the germ tubes and hyphae, being sensitive to such extremes, die. How far curing helps eradicate the deep-seated infections and protects the potato from subsequent infections is difficult to answer, but the high incidence of the disease in some of the lots of cured potatoes in the market suggests that a large percentage of dormant infections escape during curing and/or that fresh infections continue to take place.

The question as to why and how the infection originates at the ends of the potato has been answered, and in this answer can be found the explanation of why sweet potato is the major host. The relationship of the pathogen to the host is seen to be based, primarily, on the morphology of the latter, i.e., its spindle shape with pointed ends, which may carry dead tissues with dormant mycelium of the pathogen. From knowledge of the cycle of the disease in nature, it is possible to suggest an additional step in existing control measures: removal of 1 cm of both tips of the root end before or after curing.

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FACTORS AFFECTING A SEEDLING TEST FOR EVALUATING RESISTANCE OF PEA TO APHANOMYCES ROOT ROT[1]

John L. Lockwood and J. C. Ballard

SUMMARY

Miragreen pea seedlings growing in sand without nutrients were inoculated by pipetting zoospore suspensions of *Aphanomyces euteiches* beside the rows, and saturating the sand with water. Disease indices of 0–8 were based on ratings made of tops, epicotyls, and roots of plants. Several variable factors affected disease indices and standard deviations of disease indices. Disease was maximum and variability least with: depth of planting seeds, 2 cm; age of seedlings at inoculation, 4–6 days (2–5 cm high); age of culture, 4–5 days; concentration of zoospore suspension, 1.5×10^5 per ml; volume of zoospore suspension, 10 ml per 10-in. row; age of zoospores, 2–14 hours after formation; distance of placement of inoculum from row, as near as possible; number of inoculations, 1; water level in sand before inoculation, 4–6 lb. per 32 lb. of sand; saturation of sand following inoculation, saturated once; temperature of sand, 24°C. Since zoospores decreased in infectivity after 24–48 hours in sand, seedlings emerging 24 hours after inoculation should be regarded as escapes, if healthy. Of a number of interactions tested that involved these variable factors, most were not statistically significant. Consistently low standard deviations were obtained only at disease indices of 8.0.

Common root rot of pea caused by *Aphanomyces euteiches* Drechs. is, year in and year out, the most destructive disease of this crop in most pea-growing areas. Practical and effective control measures are lacking. During greenhouse evaluations of pea introductions for resistance to this disease it became evident that highly resistant lines were not available, and that precise methods would be necessary to identify lower levels of resistance. In an attempt to devise a screening test that would give consistently severe and uniform disease the effect of several variable factors on Aphanomyces root rot of pea seedlings grown in sand in the greenhouse was studied.

Materials and methods.—The susceptible pea variety used was Miragreen (Ferry-Morse Co., Detroit, Mich.). Seeds were treated for 15 minutes with 0.5% sodium hypochlorite (10% Clorox) to eliminate surface fungi such as *Rhizoctonia solani* Kühn, which frequently spread from untreated seeds, causing damping-off of large numbers of seedlings. Seeds were planted in rows in white silica sand in galvanized metal pans $13\frac{1}{2}\times15\frac{1}{2}\times4$ in. deep. Twenty seeds were planted in each of 4–6 rows in each pan. Pans were placed on a greenhouse bench at 22–26°C until emergence of the seedlings, usually in 5–6 days, at which time the pans were placed in soil temperature tanks.[2] Seedlings were usually inoculated at this stage. Nutrient solutions were not used.

Cultures of *A. euteiches*, derived from a single zoospore, were grown in 1-liter Erlenmeyer flasks containing 200 ml of maltose-peptone broth (per liter: maltose, 3 g; peptone, 1 g). Zoospores were obtained

[1]Accepted for publication February 15, 1959.
Journal article No. 2377, Michigan Agricultural Experiment Station, East Lansing.

[2]Lockwood, J. L., and J. C. Ballard. 1958. Soil temperature tanks with polyethylene film liners. Plant Disease Reptr. 42: 814-816.

TABLE 1.—*Effect of depth of planting Miragreen pea seeds on Aphanomyces root rot*

Test	Disease index[a] for indicated depth	
	2 cm	5 cm
1	7.0	4.8**
2	7.3	2.6**
3	8.0	6.3**

[a]Disease index based on a scale of 0–8, with 0 indicating healthy plants and 8 indicating dead plants. ** indicates statistical significance at 1% level.

TABLE 2.—*Effect of age of Miragreen pea seedlings at time of inoculation on susceptibility to Aphanomyces root rot*

Test	Disease index for indicated age of seedling[a]			LSD[b]
	4 days	6 days	8 days	
1	7.0	6.5	4.6	1.6
2	7.3	7.1	5.3	1.1
3	7.3	7.0	7.0	1.6

[a]Disease index based on a scale of 0–8, with 0 indicating healthy plants and 8 indicating dead plants.
[b]Least difference for statistical significance at 5% level.

in a manner similar to that of Smith and Walker.[3] After a suitable incubation period, the medium was decanted from the mycelium and replaced with 40 ml of tap water. After 2 hours the tap water was replaced by 40 ml of distilled water. About 8 hours later, zoospores were abundant in the water. Plants were inoculated by pipetting a standardized concentration and volume of the zoospore inoculum on the sand beside each row of emerging seedlings.[3,4] After inoculation of the plants, the sand was uniformly sprinkled with tap water until saturated.

Disease in tops of plants, epicotyls, and roots was estimated 8–12 days after inoculation. The most severely infected plants were usually dead by that time. Tops of plants with only the lower 1 or 2 leaves wilted were rated 1, completely wilted tops were rated 3, and intermediate stages were rated 2. Water-soaked epicotyls were rated 1, and collapsed epicotyls, 2. Slightly decayed roots were rated 1, completely decayed roots were rated 3, and intermediates were rated 2. In most of the experiments, individual plants were evaluated. The 3 ratings for each plant were totaled, and an average was obtained for each row. In a few tests, ratings of tops, epicotyls, and roots were made for the row as a whole rather than for individual plants. The disease index for a particular treatment was the average of several row ratings, usually 4. Standard deviations of disease ratings were calculated for each row as a measure of variability. Analysis of variance was applied to the disease ratings and standard deviations; row averages were used to calculate sums of squares in both instances. Where statistically significant differences are referred to, they are at the 5% or 1% level.

RESULTS.—*Depth of planting seeds.*—Two planting depths, 2 cm and 5 cm, were tested in 3 experiments. Seedlings from the shallow-planted seeds were much more severely diseased than those from the other seeds (Table 1). In one test, for example, the disease index for shallow-planted seedlings was 7.3, whereas that of deeper-planted seedlings was 2.6. Differences were statistically significant in all 3 experiments. Standard deviations for both depths of planting did not differ in tests 1 and 2. In the third test, standard deviation at the deeper level, 2.0, differed significantly from that at the more shallow level, 0.0. The latter value corresponded to a disease index of 8.0. The higher disease indices associated with shallow planting were not accompanied by decreased variability, except in test 3.

Age of seedlings at inoculation time.—Pea seedlings were inoculated 4, 6, and 8 days after planting. Plantings were spaced so that all plants were inoculated at the same time. Disease indices were higher with 4- and 6-day-old seedlings than with 8-day-old seedlings in 3 experiments, but the differences were not statistically significant in one of those tests (Table 2). Disease indices of plants inoculated 4 and 6 days after planting did not differ significantly. Disease indices for seedlings of increasing age in the test showing greatest differences were 7.0, 6.5, and 4.6. Standard deviations for the 3 ages were similar in the 2 tests for which data are available. Disease indices of plants inoculated at different ages showed no apparent relation to variability in disease.

In 2 tests there was no interaction between depth of planting seeds and age of seedling at inoculation time.

Age of culture.—The effect of age of the fungus culture on pathogenicity of the zoospores of *A. euteiches* was studied in 4 experiments. Zoospores prepared from cultures grown for 4, 8, or 12 days were adjusted to 5×10^4 or 1×10^5 zoospores per ml. Plants were inoculated with 10 ml of either standardized zoospore suspension. Zoospores from the youngest cultures gave significantly highest disease indices in all 4 tests (Table 3). For example, in experiment 1 respective disease indices for plants inoculated with

TABLE 3.—*Pathogenicity of zoospores from cultures of Aphanomyces euteiches at different ages on root rot of Miragreen peas*

Test	Disease index for cultures of indicated ages[a]			LSD[b]
	4 days	8 days	12 days	
1	7.6	4.2	2.8	1.8
2	7.8	6.4	5.9	1.6
3	7.6	6.5		0.6
4	5.8	5.1		0.7

[a]Disease index based on a scale of 0–8, with 0 indicating healthy plants and 8 indicating dead plants.
[b]Least difference for statistical significance at 5% level.

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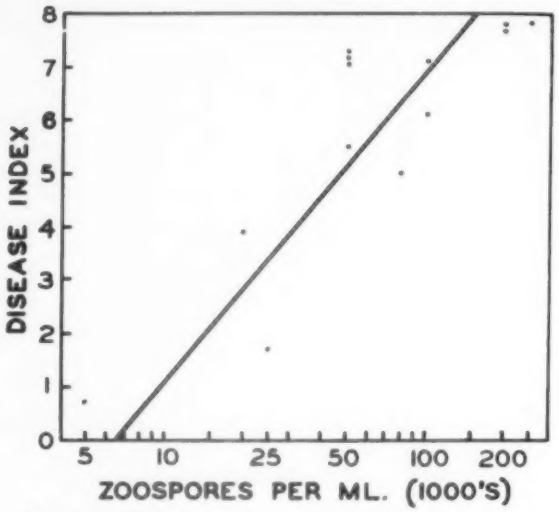

Fig. 1. Relation between disease index and concentration of zoospores of *Aphanomyces euteiches* used to inoculate Miragreen pea seedlings.

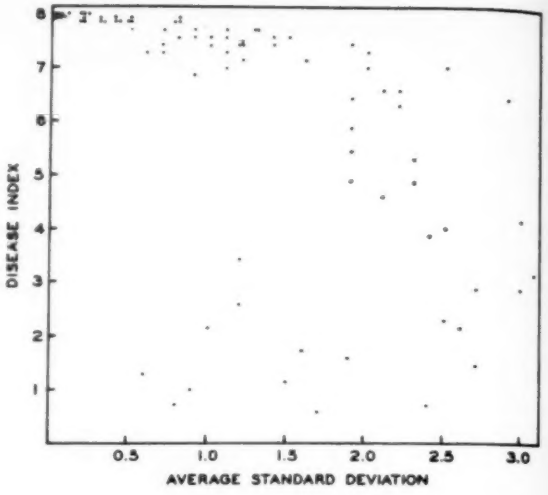

Fig. 2. Relation between disease index of Miragreen pea seedlings with Aphanomyces root rot and standard deviation of disease index.

zoospores from cultures 4, 8, and 12 days old were 7.6, 4.2, and 2.8. Respective standard deviations, available for this experiment only, were 1.2, 3.0, and 3.0, a statistically significant difference. More severe disease and less variation may be expected with young cultures. Mycelial mats less than 4 days old are extremely fragile and difficult to handle without damage.

Concentration of zoospore suspension.—Pea seedlings were inoculated with 10 ml of suspensions containing concentrations of zoospores varying from 5×10^3 to 2.5×10^5 per ml. Differences were statistically significant in 4 of 6 tests. When disease indices were plotted against logarithms of zoospore concentrations, the result was a straight line with a regression coefficient of 1.7 (Fig. 1). Correlation coefficient was 0.86, indicating a close relationship between the variates. Plotted arithmetically, the curve rises sharply, with only a slight increase in spore concentration, until concentration reaches about 5×10^4 spores per ml. Thereafter the curve flattens rapidly. In the 2 experiments lacking statistically significant differences, zoospore concentrations were 5×10^4 and 1×10^5 in one test, and 5×10^4 and 2×10^5 in the other. Fig. 1 shows that little difference in disease index could be expected with the concentrations in the former experiment. The data suggest that about 1.5×10^5 zoospores per ml should be used to obtain a consistently high level of disease.

Standard deviations were calculated from data in 4 experiments. The trend in all 4 tests was toward least variation at higher zoospore concentrations, but significant differences were lacking in 2; these had similar disease indices. High disease levels and low variability seem correlated with higher zoospore concentrations.

In one experiment there was no interaction between age of culture and concentration of zoospores.

Volume of zoospore inoculum.—The effect of applying the same number of zoospores per row in 10, 20, and 50 ml of water was tested in 4 experiments. Number of zoospores per row was 5×10^5 in some tests and 1×10^6 in others. Disease indices were significantly higher for plants inoculated with 10 or 20 ml than for plants inoculated with 50 ml in 3 of the 4 tests (Table 4). Indices of plants inoculated with 10 or 20 ml were not significantly different. In 2 experiments there was a wide difference between values for 10 or 20 and 50 ml. For example, in test 1, respective disease indices were 7.6, 6.4, and 2.4. There were statistically significant differences between standard deviations of plants inoculated with 10, 20, or 50 ml of inoculum in 3 experiments. The respective averages were 0.4, 1.2, and 2.4. In the fourth test, deviations were similar at all 3 inoculum levels. Lower volumes of inoculum tended to give most disease and least variation.

Different zoospore concentrations and volumes of inoculum were tested together in 4 experiments, with-

TABLE 4.—*Effect of volume of zoospore inoculum, with number of zoospores constant, on root rot of Miragreen pea seedlings*

Test	Disease index[a] for indicated volume of inoculum per row			LSD[b]
	10 ml	20 ml	50 ml	
1	7.6	6.4	2.4	1.3
	7.8	7.4	3.2	
2	8.0	7.5	6.4	1.0
	8.0	7.9	7.9	
3	7.9	7.7	5.9	1.5
4	7.4	7.4	7.7	0.5

[a]Disease index based on a scale of 0–8, with 0 indicating healthy plants and 8 indicating dead plants.
[b]Least difference for statistical significance at 5% level.

TABLE 5.—*Influence of sand temperature and distance of placement of inoculum from row on Aphanomyces root rot of Miragreen pea*

Inoculum placement distance (cm.)	Disease index[a] for indicated temperature				
	16°	20°	24°	28°	Av.
1.0	2.9	7.3	8.0	7.6	6.5
2.5	2.3	6.7	7.6	7.1	5.9
5.0	0.3	0.9	0.7	0.6	0.6
Av.	1.8	5.0	5.4	5.1	

[a]Disease index based on a scale of 0–8, with 0 indicating healthy plants and 8 indicating dead plants. Least differences for statistical significance at 5% level: temperature averages, 0.3; inoculum placement distance averages, 0.4; other values, 0.6. Interaction was not statistically significant.

out evidence of a significant interaction with respect to disease indices. In one test, a significant interaction occurred with standard deviations. At a concentration of 5×10^4 zoospores per ml, deviations increased with increasing volume of inoculum, a trend not evident at a concentration of 2×10^5 zoospores per ml.

Age of zoospores.—In 2 tests, seedlings were inoculated with zoospores 2 and 14 hours old. Ten hours after the medium was decanted from the cultures, most of the zoospores were 2 hours old and were actively swimming. At 22 hours they were 14 hours old, and most had encysted for the second time; some were beginning to germinate. Ten ml of inoculum containing 1×10^5 zoospores per ml was applied to each row. Neither disease indices nor standard deviations differed significantly between young or old zoospores. Differences in zoospore ages within the limits of these tests did not cause a change in severity or variability of the disease.

Period of infectivity of zoospores.—The length of time that zoospores of *A. euteiches* remained infectious after pipetting onto sand was tested in 2 experiments. A zoospore suspension was pipetted onto rows marked on sand, and the sand was saturated with water. Seedlings that had been germinated in moist paper towels were selected for uniformity and transplanted in the rows at the following intervals after infestation of sand: immediately, 6 hours, 12 hours, 24 hours, and 48 hours. Germination of pea seed was spaced to correspond with planting intervals so that seedlings were the same age when planted. Disease indices for increasing time intervals were as follows: for test 1—8.0, 7.7, 7.9, 4.8, and 2.2; for test 2—8.0, 8.0, 7.9, 7.4, and 5.3. Least difference for statistical significance was 1.2 in both tests. Disease tended to decrease in plants transplanted 24–48 hours after infestation of sand. The lower disease indices reflected fewer infected plants as well as less severe infection. In both experiments, standard deviations increased as the disease index decreased. Deviations for increasing time intervals were: for test 1—0.0, 0.7, 0.2, 2.3, and 2.6; for test 2—0.0, 0.0, 0.5, 1.2, and 2.3. Least difference for statistical significance was 1.2 for both sets of data. Seedlings emerging later than 24 hours after inoculation may have less disease and contribute to variability. Sometimes, however, late-emerging seedlings were those delayed by infection.

Distance of placement of inoculum from row.—Two experiments tested the effect of pipetting 20 ml of zoospore inoculum on a line 1 cm, 2.5 cm, or 5 cm from the row. Sand temperatures of 16, 20, 24, and 28° were used. At 24°, the respective disease indices were 8.0, 7.6, and 0.7 in the first test, and 8.0, 7.7, and 2.4 in the second (Table 5). The low values at 5 cm distance from the row differed significantly from the higher values in both experiments. Average disease indices for all 4 temperatures showed a significant difference between values for 1 cm and 2.5 cm because of the larger number of observations. Standard deviations increased with increasing placement distances, being 0.6, 1.6, and 2.0 in one test, and 0.8, 1.2, and 2.2 in the second. The 3 values in each test differed significantly. Highest disease levels and least variation can be expected with placement of inoculum as near as possible to the row.

There was no interaction between age of zoospores and distance of placement of inoculum from row.

Number of inoculations.—One experiment tested the effect of inoculating seedling rows on 2 successive days with 10 ml of a suspension containing 2×10^5 zoospores per ml. Controls were plants inoculated once or twice the same day with the same zoospore concentration. Sand was again brought to the saturation point after inoculation on the second day. All 3 treatments were rated 8.0, indicating that 1 inoculation was sufficient. Of the 260 plants in each treatment, there were only 2 plants with ratings less than 8.0 in controls, and only 4 in plants inoculated on successive days. There were no escapes.

Water level in sand before inoculation.—Two experiments were conducted on the effect of different levels of water in the sand during pea seed germination before inoculation. Two, 4, or 6 lb. of water was added to pans that had been filled uniformly with 32 lb. of sand; 7 lb. were required to saturate the sand. Water levels were adjusted each day following inoculation, and sand in all pans was saturated with water after inoculation. In one experiment, the disease index was 7.0 for seedlings germinated in sand with 2 lb. of water. This value differed statistically from indices of 7.7 for 4 lb., and 7.8 for 6 lb. In the second test, disease indices were almost identical—7.9, 8.0, and 8.0. Standard deviations, available for the first test only, were 1.2, 0.2, and 0.0, respectively, for plants in pans with 2, 4, and 6 lb., indicating significantly greater variation at the low water level. With reasonable care in watering, water levels can be kept above 2 pounds, and variation between pans to less than ½ lb. Small variations in water level do not seem critical.

There was no interaction between volume of zoospore inoculum and level of water in sand before inoculation in one experiment, nor between depth of planting seeds and level of water in the sand before inocula-

tion in one experiment.

Saturation of sand following inoculation.—The necessity of saturating sand with water after inoculation, and the effect of a second saturation on the day after inoculation, were studied in 2 tests. Respective disease indices for plants in sand unsaturated, saturated once, and saturated twice were 2.2, 7.3, and 7.5 in one test, and 3.4, 7.8, and 8.0 in a second. The larger differences were statistically significant. There was a significantly higher standard deviation for plants in unsaturated sand than in other treatments in one of the 2 experiments. Deviations for plants in sand saturated once and twice did not differ in either test. Saturation of sand after inoculation is necessary for high levels of disease and low variability, but no advantage was found in a second saturation.

One experiment showed a significant interaction between saturation of sand after inoculation and zoospore concentration. At a very low level of spores, 5×10^3 per ml, disease indices were as low in saturated as in unsaturated sand. At higher concentrations, 5×10^4 and 2.5×10^5 spores per ml, differences were marked between unsaturated and saturated sand. Standard deviations followed a similar pattern, with higher values corresponding to lower disease indices.

Temperature of sand.—The influence of sand temperature was studied by growing peas in temperature tanks at 16, 20, 24, and 28°. Seeds were germinated in sand in pans on a greenhouse bench. Pans were placed in temperature tanks 4 hours before inoculation. Two similar experiments showed that the disease developed well at 20–28°, but was very mild at 16° (Table 5). Maximum disease indices were obtained at 24° in both experiments, but statistically significant differences were lacking between 24° and 20° in one experiment, and between 24° and 28° in both tests. When more observations were included in the analysis, average disease indices for all 4 temperatures differed statistically (Table 5). These results agree with those of Smith and Walker.[3] Standard deviations, calculated for plants in sand at 20, 24, and 28°, were similar in both tests. Sand temperature between 20 and 28° is not critical.

There was no interaction between placement distance and temperature of sand in one experiment, or between age of zoospores and sand temperature in one experiment.

Relation between disease index and standard deviation.—Disease indices and corresponding standard deviations were plotted with data from the foregoing experiments to determine the relationship between the 2 variates (Fig. 2). Each of the 87 points plotted is the average value of 4 rows of seedlings. For points below a disease index of 7.0, the correlation coefficient was 0.20, indicating no significant relation between disease index and standard deviation. Above 7.0, however, the correlation coefficient was 0.84, indicating a close relation between the 2 variates. Similarly, in individual experiments there was no evidence of a relationship between disease index and standard deviation when disease indices were below 7.0. Increasing disease indices usually became correlated with decreasing standard deviations when the disease index increased from a value below 7.0 to one near 8.0. Uniformity can be made maximum and most consistent by attaining maximum severity of disease.

Discussion.—The value of a screening method lies in its efficiency and the reliability with which it can be used to distinguish differences at a desired level of precision. In the case of Aphanomyces root rot of pea, only low levels of resistance have thus far been found, and a screening method must be sufficiently sensitive to separate slightly resistant from susceptible material. A test too severe might eliminate material with low resistance; one too mild might result in selection of susceptible plants. Variation in disease within a test would be chaotic. The problem becomes particularly important in testing segregating breeding lines where individual plants must be selected.

The foregoing experiments were carried out in an effort to determine the important variable factors in the Aphanomyces root rot test. Of the 12 variable factors tested, 10 affected disease index. Of 11 variable factors for which standard deviation data are available, 8 showed significant differences. The results stress the importance of careful standardization of technique in evaluating peas for root rot resistance. Even when conditions were carefully controlled, variation in disease was sometimes evident. It is possible that other factors, such as air temperature and humidity (which could not be controlled in these experiments), might be important sources of variation from test to test.

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BOTRYTIS CINEREA THE CAUSE OF GHOST-SPOT DISEASE OF TOMATO[1]

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SUMMARY

Studies were conducted on the causal agent and conditions required for the development of the ghost-spot disease of tomato fruits. Attempts to reproduce the symptoms by exposing tomato fruits to various environmental conditions and by mechanical injury produced evidence against a physiogenic cause. Inoculation of immature fruits with *Botrytis cinerea* produced symptoms undifferentiable from those occurring naturally in the field. Fruits inoculated with a spore suspension of *B. cinerea* and incubated 8–12 hours at 16–24°C in the presence of high humidity formed necrotic specks similar to those found in the center of ghost spots. If these fruits remained at this temperature and high humidity, the Botrytis gray mold rot developed. If, after incubation at 16–24°C, fruits were left 6–24 hours at 30–35°C, a solid white disk often appeared around the necrotic spot, or if incubated at 38–40° C, the ghost-spot symptoms developed. Tomato fruits 1.5–3.0 cm in diameter became heavily spotted when inoculated; those less than 1.5 cm showed large necrotic areas; those larger than 3 cm developed few or no ghost spots. Histological studies indicated that the halo surrounding the necrotic speck was formed by air spaces between the epidermal layer and the collenchymatous tissue. Stained tissue at the necrotic specks showed the presence of appressorium-like material, suggesting a penetration of the host by the fungus. Increase in the thickness of the walls of the epidermal cells was observed, as well as trichomal penetration by *B. cinerea*.

The ghost-spot disease of tomato has become a serious problem throughout most of the southern tomato-growing areas of Florida. The production of this disease has been a very controversial topic, but *Botrytis cinerea* Pers. is at present doubtfully accepted by American investigators (6, 11, & 15) as the causal agent. Bryan (2) first described the ghost-spot disease in 1933 and suggested that insects feeding on the tomato fruits were the causal agents. Walton (16), in 1937, believed that the disease was the result of the lens action of sunlight through water droplets on the fruits. Read (13), in 1937, reported that *B. cinerea* was the causal agent of ghost-spot in England, and Ainsworth, Oyler, and Read (1) supported Read's work.

Ramsey (10, 11), in 1938 and 1939, Harrison (7), in 1938, in the United States; and "K. M. Graham (Conners 3, p. 83), in 1953, in Canada." reported ghost spot on tomato fruits, but did not comment on the nature of the causal agent. Doolittle (6), in 1948, included ghost spot among diseases whose origin was not definitely known. Ramsey, Wiant, and McColloch (11), in 1952, stated that no fungus or other organism has been found associated with ghost spot in the United States, and that the actual causes in this country have not been proved.

Darby (4), in Florida in 1955, supported Read's hypothesis with statistics from fungicidal tests used to control the fungus *B. cinerea*, but failed to reproduce the disease in direct inoculation with this fungus.

The investigations reported herein were conducted in field plots, greenhouse, and laboratory to correlate the presence of the causal agent with the disease and to ascertain the conditions required for development of ghost-spot disease on tomato fruits in Florida.

PHYSIOLOGICAL STUDIES.—The spotting of tomato fruits was often found in fields where gray mold caused by *B. cinerea* had occurred earlier in the season, but, often, the 2 diseases occurred simultaneously in the same field. In some cases, fruits marked with ghost spots also had a rot caused by the gray mold fungus.

In contrast to the common gray mold diseases, which usually occurred either at the blossom-end or stem-end portions of the fruits, this disease frequently occurred on the shoulders and lateral surfaces of fruits.

Ghost spot of tomato in Florida was found to be most prevalent in March, April, and May, and after periods of heavy dews or night rains followed by warm, sunny mornings. When atmospheric conditions were of this type moisture was most likely to be deposited on fruits at night and evaporated by the early morning sun. The diseased fruits were found most frequently on the exposed portions of the plant, and on that part of the fruit facing the direct rays of the sun. Ghost spots invariably formed on the upper halves of immature fruits of 1.5–3.0 cm in diameter, and were sometimes observed on the lower halves of larger immature fruits, and occasionally on coloring mature fruits.

The required atmospheric conditions mentioned above, the failure to isolate an organism from the spots, and the absence of decay have suggested that the cause might be physiological. Walton (16) reported that ghost spots were the result of the action of sunlight through water droplets on the surface of the tomato fruits. A series of experiments were conducted to determine whether some environmental conditions might be the cause.

Effect of water droplets and sunlight.—In a bench in a greenhouse, 3 rows of tomato plants were dis-

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tributed so that equal numbers of plants in each row received either direct sunlight, shade, or semishade. The outer two rows of plants (104 fruits) were sprayed with water 3 times a day; as a control, the middle row of plants (49 fruits) received water only from the soil. In 20 days, ghost spots were found on 3 fruits that had received aerial water and 2 that had not. The diseased fruits appeared irregularly throughout the experimental area, and there was no correlation with direct or indirect sunlight.

Effect of low and high temperature.—Fruits to which droplets of distilled water had been added were exposed to a temperature of 40°C, obtained with a 500-watt photoflood lamp, until evaporation was total. Before the droplets were added, 5 fruits were stored 1 hour at 4°C, and 5 others were stored 15 minutes at 40°C. No ghost spots or necrotic specks were obtained.

Effect of ultraviolet rays.—Ten fruits with drops of distilled water on them were exposed to a General Electric 8-watt germicidal tube, alone or with a 500-watt photoflood lamp, until the water evaporated. Neither ghost spots nor necrotic specks developed.

Effect of mechanical injury.—The possibility was tested that the spots could have been produced by minute mechanical injury. Fruits were injured by needles ½ and 1½ mm long, by carborundum, and also by rubbing the surface with cheesecloth to damage the trichomes or epidermal cells. These experiments were conducted on both attached and detached fruits, but no ghost spots or necrotic specks developed.

Effect of concentrated sunlight.—Following Walton's hypothesis (16) that the spots were produced by sunlight focused on the surface of the fruit by water droplets acting as lenses, 24 tomato fruits were exposed ½, 1, 1½, and 2 minutes. Twelve fruits thus treated were sprinkled with tap water; 2 were not sprinkled. A blister-like burning of the epidermal cells was observed, but no typical ghost spots or necrotic specks were detected.

Pathogenic studies.—Read (12) stated that the occurrence of ghost spots on tomato fruits, as observed by English growers, was closely associated with gray mold rot of tomato caused by *B. cinerea*. He inoculated immature tomato fruits with spores of this fungus and obtained symptoms similar to those found in the field. Ainsworth and co-workers (1) supported Read's findings, and further reported that *B. tulipae* (Lib.) Lind., *B. allii* Munn, and *B. fabae* Sard. produced similar symptoms on tomato fruits. Darby (4) was unable to reproduce this disease experimentally with *B. cinerea*.

Botrytis species have been known for some time to cause spotting on plants from which no internal mycelium could be detected, nor could any fungus be isolated. All attempts of the writers to isolate parasitic organisms from ghost-spotted material have been unsuccessful. Correlation of the ghost-spot phase and the Botrytis fruit rot of tomato suggested a striking similarity to the foliage and corm infection of gladiolus, described by Dimock (5) as caused by *B. gladiolorum* Timmermans; a leaf spotting and neck rotting of onions, described by Segall (14) as caused by *B. allii* Munn, and a spotting of Cymbidium flowers, described by Jefferson as caused by *B. cinerea*.

Materials and methods.—Pure cultures of *B. cinerea*, isolated from infected tomato fruits, leaves and stems, were used as sources of inoculum. Isolations were made from infected tomato plant parts obtained from West Palm Beach, Immokalee, Belle Glade, Bradenton, Fort Pierce, and Gainesville, Florida. No differences in pathogenicity could be detected among these isolates.

Fruits were inoculated by allowing droplets of spore suspension to drip from a sterilized camel's-hair brush onto the tomato fruits, by dipping the fruits in the spore suspension, and by spraying the spore suspension on the fruits with an atomizer. The last was the method most commonly used. After inoculation, the fruits were incubated in wide-mouthed flasks, containing a small amount of water to maintain a high humidity, and plugged with non-absorbent cotton.

Attached tomato fruits, abscised fruits, and fruits on abscised branches were used in the pathogenicity tests. When detached fruits were tested in the laboratory, they were incubated in moisture chambers containing moistened towels to maintain a high humidity. The peduncles of the fruits and the detached branches of fruits were placed in a water bath to maintain turgidity.

Inoculation tests.—In the first experiment, 166 detached immature tomato fruits, 0.5–5.0 cm in diameter, were inoculated with spore suspensions of *B. cinerea*. The fruits thus treated were then incubated 12 hours at 16° and 24°C. After 8–10 hours, most of the inoculated fruits at both temperatures showed brown necrotic specks similar to those observed in the center of the ghost spots in the field (Fig. 1-A). Amount of penetration by the fungus and fruit responses to penetration were observed to be directly proportional to the size of the fruits. Fruit less than 1.5 cm in diameter became blistered at the penetration points, and later developed brown necrotic areas covering the smallest fruits almost completely. Fruits 1.5–3.0 cm in diameter showed the greatest number of necrotic specks, whereas fruits 3–5 cm in diameter developed necrotic specks that were smaller and fewer in number than those previously described. After incubation, one half of the inoculated fruits were exposed to sunlight for 10 hours, and again incubated at 16° and 24°C. The other half were incubated at 16° and 24°C without exposure to sunlight. Halos developed on a single fruit in the group exposed to the sun.

In a second experiment, 10 ripe tomato fruits showing red color were inoculated by the drip method with spore suspension of the fungus, and then incubated 7 days in moist chambers at 24°C. The characteristic fruit rot caused by the fungus was observed after 5 days in 9 of the 10 fruits, but no ghost spots could be detected.

In a third experiment, 16 fruits attached to 5 plants

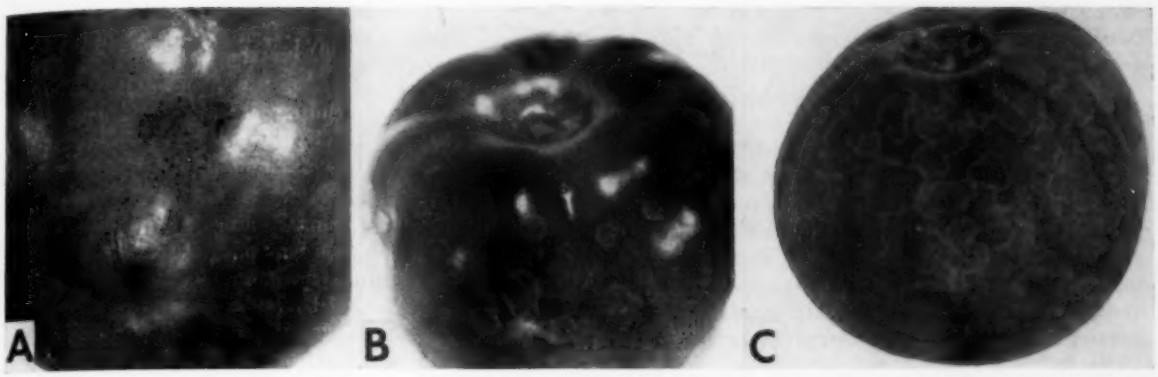

Fig. 1. **A)** Brown necrotic specks on tomato fruit produced by germinating conidia of *B. cinerea*. **B,C)** Ghost spots on artificially inoculated fruits.

growing in 8-in. clay pots were inoculated by spraying with a spore suspension of *B. cinerea*. They were covered with wide mouthed bottles, incubated for 20 hours at 24°C. and retained at the same temperature for 7 days. For chlorophyllic activity, the plants received 12 hours of artificial light per day. The fruits on a control plant were treated with distilled water instead of a spore suspension. Another group of 49 fruits were treated under the same conditions, but after 20 hours of incubation were transferred to a bench in the greenhouse. No characteristic ghost spots with halos were formed, but a large number of necrotic specks surrounded by whitish disk-like areas developed on the fruits remaining at 24°C following inoculation. Nine of the 49 fruits transferred to the greenhouse showed typical ghost spots with halos. A total of 27 fruits developed Botrytis fruit-rot after 4 days.

In a fourth experiment, 127 fruits on pot-grown plants were inoculated by the drip and spray methods, covered with Cellophane bags, and placed overnight under a mist sprayer. Controls were 30 fruits, watered with tap water. During this experiment, a portion of the greenhouse was covered with a thick coat of white paint to protect the plants from the direct rays of the sun. After 14 days, 11 inoculated and 3 control fruits growing on plants in the unshaded portion of the greenhouse had developed ghost-spot symptoms. With few exceptions, brown necrotic specks similar to those found in the centers of ghost spots in the field were formed on all of the inoculated fruits. The cells surrounding the necrotic specks formed whitish disk-like symptoms, except in fruits showing halos.

In a fifth experiment, 26 plants with a total of 70 fruits were selected at random in a tomato field plot. The fruits were atomized with a spore suspension, incubated 12 hours in wide-mouthed flasks, and then removed and exposed to the sunlight. Three plants carrying 10 fruits were used as uninoculated controls.

After 7 days, 41 of the 70 inoculated fruits showed ghost-spot symptoms (Fig. 1-B,C), and the control fruits showed no symptoms. The first symptoms observed in the field, necrotic specks surrounded by a light-brown area, were detected as soon as the spore suspension droplets had evaporated. The typical ghost-spot symptoms showing the halo were noticeable 48 hours after inoculation.

Factors influencing the formation of ghost spots.—Preliminary inoculation studies conducted with *B. cinerea* in the laboratory and greenhouse yielded negative results, but inoculated fruits on plants grown in the field became spotted. Several factors, such as fruit size, variety, and temperature, appeared to be correlated with the formation of ghost spots. To test some of these factors, the following experiments were conducted.

1) *Fruit size.*—The sizes of the fruits of field-grown tomato plants were recorded at time of inoculation. The diameter of the fruit was determined by taking averages of 2 cross measurements at the blossom-end of the fruit. The fruits were inoculated by the methods used in the field experiment.

Ghost spots developed on 17 of 28 inoculated fruits less than 1.5 cm in diameter, 20 of 28 inoculated fruits 1.5–3.0 cm in diameter, and 4 of 14 inoculated fruits greater than 3 cm in diameter. Fruits less than 1.5 cm in diameter showed fewer spots, and usually developed necrotic areas where the spore suspension droplets had been. Fruits measuring 3 cm in diameter or more did not become spotted if the cuticle appeared glossy and the fruits dark green in color. Very young inoculated fruits never reached maturity, and, if less than 1 cm in diameter, remained small and deformed.

2) *Variety of tomato.*—The varieties Jefferson, Manasota, Manalucie, Manalee, Marglobe, and Grothens Globe "K" strain were grown side by side in a field. When inoculated with spores of *B. cinerea*, none of the 6 tomato varieties showed any resistance to ghost spot, and there was little difference in the amount of spotting that occurred.

3) *Temperature.*—When inoculations were conducted at a time when the night temperature was below 21°C for more than 4 hours, the percentage of ghost spotting was high. Later in the season, inoculated fruits exposed to temperatures between 21° and 24°C needed more than 8 hours in this range for production of ghost spot. Few or no ghost spots were produced after 12 hours of incubation at temperatures above 25°C. It was also observed that the percentage of

ghost spots was higher when the inoculation was followed by a warm sunny morning instead of a rainy morning.

The germination of *B. cinerea* spores in droplets of distilled water on glass slides retained during these experiments indicated that with temperatures above 20°C at night during incubation of the inoculated fruits, germination of spores decreased in direct relationship to an increase in temperature. With temperatures between 20° and 27°C during the incubation period, the higher the temperature, the fewer spores germinated. At temperatures above 29°C, no germination of spores was observed on glass slides.

Conidia of *B. cinerea* were dusted onto the surface of PDA (potato-dextrose agar) in Petri plates and incubated 12 hours at 25°C, which allowed the spores to germinate and produce some mycelium. Five plates were placed 12 hours at each of the following temperatures—24°, 28°, 32°, 36°, 40°, and 44°C—and then placed 48 hours at 24°C. No growth occurred at temperatures of 32° and above.

In a greenhouse inoculation experiment where a part of the inoculated plants were shaded and the remainder exposed to direct sunlight, it was noted that ghost spots occurred only on fruits in the unshaded portion of the greenhouse. The temperature for 5–6 hours during the day was about 40°C around the fruits in the unshaded area, and 33°C around fruits in the shaded portion.

Experiments were devised to determine the effect of temperature on ghost-spot production. Five pots of tomato plants with 1 cluster of fruit per plant (a total of 13 fruits) were used. The fruits were inoculated with conidia of *B. cinerea* and incubated for 12 hours at 20–24°C and then placed at 38–40°C for 12 hours. The fruits were left in wide-mouthed jars containing water, and the soil in the pots was thoroughly wet. Ghost spots were abundant on 6 fruits, 2 fruits had faint halos, and 5 had no halos. When the experiment was repeated, halos were abundant on 4 of 15 fruits inoculated. The spotted fruits were on plants that remained very turgid at 38–40°C; plants slightly wilted were not spotted.

In a third experiment, excised clusters of fruits were placed in moist chambers with the cut portion of the stems submerged in water at the bottom of the chambers. Each moist chamber contained 70 fruits, 2–4.5 cm in diameter, and mostly 3.5 cm. The fruits were sprayed with a suspension of *B. cinerea* spores and then were incubated 12 hours at 22–24°C, at which time small necrotic specks were discernible. There appeared to be a very slight swelling surrounding these necrotic specks. The fruits were then incubated at 33–35°C, 38–40°C, and 42–44°C. At 7 hours there were ghost spots on the fruits at 38–40°C and, at 24 hours, 42 of these fruits were heavily spotted (15–75 halos each), 20 had fewer halos (1–15), and 8 fruits had developed no halos. The halos were typical, except that they were smaller than those that appeared on attached fruits (0.5–2.0 mm in diameter). No halos formed on the inoculated fruits at 42–44°C, which were injured by the high temperature, showing scald symptoms. Many of the fruits incubated at 33–35°C formed whitish disks around the necrotic speck, but no halos appeared.

When this experiment was repeated, results were similar at 33–35°C and 42–44°C, but only 12 fruits at 38–40°C showed halo symptoms.

4) *Pectinase.*—Attempts were made to reproduce the ghost-spot symptoms using purified commercial pectinase (Nutritional Biochemical Corporation). A dilution series of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} g/ml was used, and 10 detached fruits ranging from 2½ to 5 cm in diameter were injured with a needle ½ mm long, sprayed with each dilution, and incubated 7 days at 24°C. At 44 hours, no halos were observed, but a light yellowish area surrounding the point of injury was observed with the dilutions 10^{-3} and 10^{-4}.

A second experiment was conducted with a filtrate obtained by germinating spores of *B. cinerea* in sterile distilled water overnight, and then filtering through a Seitz filter. Thirty fruits on detached branches, retained in water baths, were injured with a needle ½ mm long, sprayed with the filtrate, and incubated at 24°, 28°, and 32°C. Twenty fruits from plants grown in 8-in. clay pots in the greenhouse were inoculated with the same procedures. At 7 days, none of the inoculated fruits showed ghost-spot symptoms or reaction to filtrate treatment.

Histology.—Ainsworth and co-workers (1) studied penetration of the tomato fruit by the fungus *B. cinerea* and concluded that a direct penetration occurred, resulting in the formation of granular cytoplasm inside the infected cells, followed by the collapse of adjacent cells to form the necrotic specks. They could not detect any trace of mycelium in material fixed in weak Fleming's solution 4 days after inoculation. They also stated that the halo surrounding the necrotic speck was caused by a layer of air between the epidermis and the underlying tissue.

Darby (4) made paraffin sections of the ghost spot and detected no evidence of an infection peg or mycelium in the necrotic specks. He reported that the necrotic areas were deeply stainable with safranin, suggesting lignified or cutinized cell walls, or tissue of basic character. He did not mention the nature of the halo surrounding the necrotic speck.

Materials and methods.—Preliminary attempts with the paraffin method indicated that it was not suited for this type of material, and the sectioning of fresh material therefore became necessary. Sections about 20 μ thick were cut with a freezing microtome and stained with ruthenium red, phloroglucinol-hydrochloric acid, and Sudan IV to determine the response of the ghost-spotted tissue to the action of specific stains. The staining of freehand sections and peeled-off epidermal tissue was investigated. Fine sections were cut with a razor blade and peeled-off epidermal tissue was taken from artificially and naturally spotted fruits. The sections were mounted on a glass slide, flooded with lactophenol–cotton-blue dye, as described

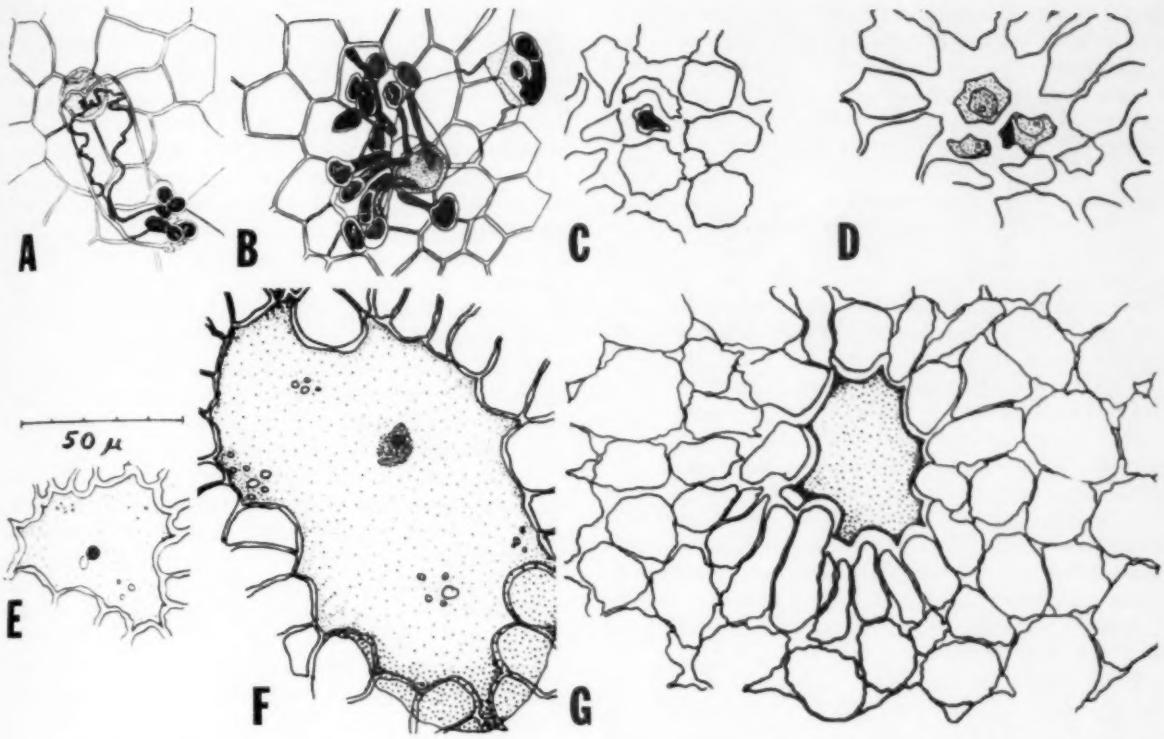

Fig. 2. A-G) Photomicrographic drawings. **A)** Germ tubes penetrating a glandular trichome into the fruit tissue. **B)** Germ tubes penetrating fruit at base of broken trichome. **C,D)** Sections through necrotic specks of ghost spots showing black penetration peg of *B. cinerea* conidium in the early stages of infection. **E)** Round, crystal-like structures in tissue of necrotic speck. **F)** Section through older necrotic speck, showing infected area and thick-walled cells adjacent to necrotic tissue. **G)** Thick-walled cells surrounding the necrotic speck, elongated radially and aligned in a peripheral fashion.

by Riker and Riker (13), covered, warmed gently until fumes were given off, and then examined. This modification of the lactophenol–cotton-blue technique kept the spores from being washed from the surface, and proved to be the best method for histological studies of ghost spots.

Results.—With ruthenium red, a specific stain for pectin, an increase in the intensity of the characteristic red color of pectin was observed in some cases, indicating an accumulation of a pectic compound in the middle lamellae of the cells forming the ghost spot. This condition was by no means constant. Phloroglucinol-hydrochloric acid, a specific stain for lignin, did not stain the damaged areas in 15 minutes. A very deep red coloration was obtained in the cell wall enclosed in the ghost spot with Sudan IV, indicating an accumulation of cellulose and, possibly, pectin.

Stained free-hand sections and peeled-off epidermal tissue indicated that the halo was caused by air spaces between the epidermal cells and the uppermost layer of collenchyma tissue. These observations coincide with those of Ainsworth and co-workers (1). The disk-like symptom commonly obtained when inoculations were conducted in the greenhouse was found to contain a larger number of separated air masses between the epidermal and the collenchyma tissue. The epidermal cells enclosed inside the halo had walls thicker than the cell walls of healthy tissue of the same fruit.

Peeled-off epidermal tissue showing very young necrotic specks was stained with improved lactophenol–cotton-blue, and consistently showed a fungus-like material that resembled the penetration peg of the *Botrytis* spore appressorium (Fig. 2-C,D). This material was less frequently observed in ghost spots showing the surrounding halo, indicating that the fungus disappeared from the older spots. Round crystal-like structures were also commonly observed in the tissue of the necrotic specks (Fig. 2-E).

In the formation of the necrotic specks, a single cell was observed to be infected by the fungus, and 3 or more of the surrounding epidermal cells appeared to have thick walls. In the development of the spot, there followed an increase in thickness of the cells farthest away, and an increase in size of the necrotic speck caused by the dissolution of the cell walls of the surrounding brownish-colored cells (Fig. 2-F). The collapse of the cell walls, along with the coalescence of the granulate protoplasts, developed into an inward pulling force that caused the cells surrounding the necrotic speck to become elongated and to align

in a peripheral fashion, thus giving the speck an appearance of a many-pointed star (Fig. 2-G).

The ghost spot itself was found to have an elevated central portion, in which the necrotic speck was located. These necrotic specks were somewhat sunken below the adjacent epidermal cells, and were composed of the epidermal cells plus the first 2 layers of the collenchyma tissue beneath.

Penetration of the host by the fungus B. cinerea.—Typical ghost-spot symptoms were obtained by inoculating immature tomato fruits with the fungus *B. cinerea*, and experiments were conducted to determine the method of penetration and the development of the fungus in the fruit.

Conidia of *B. cinerea* were observed to germinate in 3–4 hours in a film of water, at temperatures of 17–24°C. Temperatures above and below this range retarded or arrested germination of the spores. The germ tubes were rounded and swollen at the ends, forming the appressoria, which secured the germ tube to the host tissue. Within 24 hours of inoculation the germinated spores were easily detached from the host tissue and could be washed from the surface, leaving in place a very thin, deep-blue material stainable with lactophenol–cotton-blue.

Vertical sections through the epidermis showed that the appressoria produced pegs that penetrated into the tissues of the fruit, apparently through the cuticle and the primary wall of the epidermal cell. When this happened, the penetrated epidermal cell began to show changes, especially in the nature and appearance of the cytoplasm. The cytoplasm changed from typical vacuolate to a granular condition, and the color of the cell changed from typical hyaline-greenish to yellowish-brown. The granular appearance of the cytoplasm was due to the accumulation of droplets of oily material. This change was found not only in the cells invaded by the fungus but also in those epidermal and collenchymatous cells surrounding these invaded cells.

The specks produced on inoculated fruits in the greenhouse were similar to those observed on naturally infected fruits in the field. The specks formed on detached fruits in the laboratory were observed to be larger, but apparently morphologically similar.

Some conidia of *B. cinerea* germinated on the exposed upper cell of the glandular trichome of the tomato fruit, producing germ tubes that penetrated the length of the trichome and down into the fruit tissue (Fig. 2-A). Penetration at the base of broken trichomes was also observed (Fig. 2-B). A yellowish-brown color was noticeable at the basal cell of the trichome when those spores were observed to germinate. No association was detected between trichomal penetration and ghost-spot formation.

DISCUSSION.—Ghost-spot symptoms were produced in the laboratory and in the field with spores of *B. cinerea*. Penetration-peg-like structures were constantly found associated with the necrotic specks of the ghost spots formed naturally and by inoculation. These confirmed the work of Read (11), who stated that *B. cinerea* was the causal agent in England.

It was observed that cells adjacent to the invaded cells at the necrotic specks were killed by penetration of the fungus. The invaded and uninvaded cells were probably killed by the action of a toxin secreted by the fungus. The action of the toxin is not known; however, its existence depended upon the penetration or presence of the fungus. High temperatures of 30–35°C did not stop cell killing at the point of penetration as long as the fruit had been penetrated by the fungus, although investigations showed that these temperatures killed the fungus.

Penetration by the fungus or the production of an enzyme by germinating spores caused the initial injury to the fruit. The observed thickness of the epidermal cell walls enclosed in the ghost spots could result from the dissolution of the complex pectic compounds by the enzymatic activity of the fungus. The breakdown of these pectic compounds apparently resulted in freeing a large quantity of smaller molecules of pectic compounds, thus accounting for the increase in coloration obtained in staining with ruthenium red and Sudan IV.

Enzymatic action alone did not produce halos; consequently, combinations with other factors are suggested, such as transpiration, interference with the metabolic processes of the host, relatively high temperature, and the period that the active growing fungus has been in the host tissue. When inoculated fruits were incubated at 25–36°C, no typical halos were formed, although the amount of enzyme produced could have been at a maximum.

Under experimental conditions, ghost spots were produced on tomato fruits at high temperature and high humidity, indicating that these factors were initiators of the conditions required for the development of the halo surrounding the necrotic speck. Direct sunlight brought about higher temperatures around the exposed fruits, killed the penetrating fungus, and caused denaturation of the enzyme secreted by the fungus, thus forming the air spaces observed in the ghost spots. It is probable that high temperatures denatured the enzyme secreted by the fungus and aided in the dissolution of the middle lamellae. The contracting force exerted by the collapse of the cells forming the necrotic speck aggregated the air masses at the periphery of the spots, resulting in the formation of the halos.

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RESPONSES OF ROOT-LESION NEMATODES, PRATYLENCHUS BRACHYURUS AND P. ZEAE, TO VARIOUS PLANTS AND SOIL TYPES[1]

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SUMMARY

Thirty-three plant species and varieties were tested as to relative suitability for infection and reproduction of *Pratylenchus zeae*. Of these, 4 were classified as very favorable, 6 favorable, and 23 not favorable. Of 30 plant species and varieties tested with *P. brachyurus*, none were classified as very favorable, 9 as favorable, and 21 as not favorable. Reproduction of *P. brachyurus* on host plants over 5 months indicates that populations can become high on corn, soybean, and peanut plants, but low on oats and crotalaria. Norfolk sandy loam was found to be the most favorable soil type for the infection and reproduction of root-lesion nematode, *P. brachyurus*, on suitable host plants. Cecil clay loam was the least suitable and Portsmouth loam was intermediate. Migration of root-lesion nematode, *P. zeae*, was influenced by soil type and the presence of a suitable host plant. Maximum nematode migration occurred in the Norfolk sandy loam planted with corn. Shortest distance of migration was in the Cecil clay soils, whereas distance was intermediate in Portsmouth loam. In the absence of host plants, little or no migration occurred in any of the soil types.

Root-le-ion nematodes, *Pratylenchus* spp., are very important pests of major crop plants such as tobacco, cotton, corn, and peanuts in the southeastern United States. In observations and studies on the brown root rot of tobacco, Lehman (4) and Graham (2) indicated the destructive potential of members of this genus.

Efforts to control these nematodes depend on such practices as crop rotation, use of resistant or tolerant varieties, cultural practices, and soil fumigations. Though effective use of crop rotation and selection of resistant varieties are based on an understanding of host susceptibility, there is little information on this in the literature. Also, little is known of the ecological factors affecting the reproduction and survival of *Pratylenchus* spp.

Investigations reported herein deal with 2 species of root-lesion nematodes, *P. brachyurus* (Godfrey, 1929) Filipjev & Stekhoven, 1941, and *P. zeae* Graham, 1951. The objectives were to study: 1) the relative suitability of important crop plants grown in the southern states as hosts for *P. brachyurus* and *P. zeae;* 2) the effect of soil type on the development and survival of *P. brachyurus;* and 3) the effect of soil type on migration of *P. zeae* in the soil, either with or without host plants.

MATERIALS AND METHODS.—*Maintaining populations, preparation of inoculum, and assay techniques.*—Single-species cultures of *P. brachyurus* and *P. zeae* were started by adding picked specimens to nematode-free soil. Stock cultures in 6-in. clay pots were increased and maintained on corn, a highly suitable host for both species. The host plants were grown in Norfolk sandy loam.

Nematode inoculum for each experiment was pre-

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pared from infected corn roots thoroughly washed to remove soil particles. The roots were then cut with scissors into pieces about ⅛–¼ in. long and fragmented for 20 seconds in a Waring Blendor containing about 160 ml of water. Excess water was drained off by pouring the mixture into a 325-mesh sieve, and the nematodes and root debris remaining were washed into a Baermann funnel. Wet-strength tissue supported on aluminum screens, as described by Anderson and Yanagihara (1), retained the root fragments but allowed the nematodes to move downward and settle in the stem of the funnel. Nematodes were collected in a few ml of clear water, and the number of nematodes per ml were counted. Prescribed numbers of nematodes per volume of water were obtained by dilution. A 25-ml pipette fitted with a short piece of rubber tubing and metal clamp was used for measuring out aliquots of nematode suspensions.

Host-range studies.—Forty-two species and varieties of plants were tested under greenhouse conditions to determine their suitability as hosts for either or both of the species of nematodes. Six-inch clay pots were filled three-fourths full with nematode-free soil, and about 200 nematodes were added to each pot by distributing the nematode inoculum over the soil surface. Test plants, propagated from seed, cuttings, or eyes in 2½-in. clay pots, were transplanted to the 6-in. pots, which were then filled with nematode-free soil. Each test plant or plants in 6-in. containers were replicated 5 times and allowed to grow 60 days at temperatures ranging from 70 to 80°F. Moisture and fertility conditions were maintained at levels optimum for plant growth.

At the end of the test period, nematode populations were determined for each test plant. The soil was removed from around the roots by washing under a gentle stream of water. Total weights of roots, pressed out with paper towels, were recorded. To obtain a representative sample of roots for nematode assay, the entire root system from each pot was first cut into small fragments (⅛–¼ in.), and the fragments thoroughly mixed. A 5-g subsample was then further fragmented in a Waring Blendor, sieved, and placed in the Baermann funnel, as previously described. After 6 days, samples were drawn off and counts made in transparent plastic dishes 7.4 × 3.2 cm and 1.3 cm deep. The undersides of the dishes were etched along the long axis with parallel lines 3 mm apart to facilitate counting with the aid of a stereoscopic microscope (36×). If populations exceeded 500 nematodes per sample, dilutions were made before counting.

Nematode infection and reproduction in relation to soil types.—The four representative soils used in this experiment were 1) Norfolk sandy loam—a light, yellowish-brown, well-drained soil associated with loose sand subsoils and located in the upper and middle coastal plain of North Carolina; 2) Portsmouth loam—a dark, gray to black soil, poorly drained and associated with sandy loam to friable clay-loam subsoils in the lower, middle, and upper coastal plain of North Carolina; 3) Cecil clay loam—a reddish-brown, friable, slightly gravelly clay loam associated with red clay subsoils and derived from acid crystalline rocks in the Piedmont region of North Carolina; and 4) sand of river-bed origin (3).

The soils were screened through a ½-in. wire mesh to remove coarse materials and debris. Half of the soil or sand for each type used was treated with methyl bromide at a rate comparable to 3 lb. per 100 sq. ft.; the other half was treated with D-D (1,3-dichloropropene, 1,2,-dichloropropane) at a rate comparable to 40 gal./acre. The fumigants were applied in an attempt to provide a differential in the biological components of the soil so that it might be possible to study the influence these treatments might have on the nematode populations.

Two species, strawberry (*Fragaria chiloensis* Duch. var. *ananassa* Bailey) and cotton (*Gossypium hirsutum* L.), were used as indicator plants. Nematode-free strawberry plants produced from runners were propagated in nematode-free soil until the roots were well established and then transplanted into the 4 soil types; cotton seeds were sown directly.

A water suspension of about 500 adults and larvae of *P. brachyurus* were added to each pot at the time of planting. The inoculum, placed about 2, 3, and 4 in. below the top of the pot, was distributed over the entire area of each pot at those depths.

This experiment was conducted twice. Each treatment (crop, soil type, chemical treatment combination) was completely randomized, and replicated four times under greenhouse conditions with temperature ranging from 70 to 80°F. Water and fertilizer were added to each container as needed to maintain optimum growth of the indicator plants. After a 3-month growing period, roots of the indicator plants were assayed for nematodes according to the procedures described.

Nematode migration as influenced by soil type and presence of host plant.—To eliminate nematodes, the Norfolk sandy loam, Portsmouth loam, and Cecil clay soils used were fumigated with methyl bromide gas at the rate of 3 lb./100 sq. ft. 1 month before the test was initiated. To provide optimum drainage, a 1-in. layer of gravel was placed over the bottoms of galvanized iron tubs with holes drilled in the bottoms. The various soil types were then placed in the tubs, surrounding a metal cylinder, 1 in. in diameter, that extended from the top of the container to the gravel. This centrally located cylinder was filled with bits of corn roots infested with large populations of *P. zeae* mixed in soil like that surrounding the cylinder. Nematode-free soil was placed in the upper inch of the central core to avoid surface washing of nematodes. The containers were tapped lightly to settle the soil, and the cylinder was then removed, leaving the nematodes in a central core of soil. A 2½-in. inverted clay pot was placed over the top of the infested central core to reduce direct contact via water. Four replicates of each of the 3 soil-type treatments were seeded with corn. Eight plants were allowed to develop in

TABLE 1.—*Plant species and varieties tested for suitability as hosts to species of root-lesion nematode, Pratylenchus zeae and P. brachyurus,*[a] *in a 2-month test*

Name	Plants tested	Variety	Mean number of nematodes per pot[b]	
			P. zeae	*P. brachyurus*
Alfalfa	(*Medicago sativa* L.)	Atlantic	7	38
Alfalfa	(*Medicago sativa* L.)	Kansas common	0	73
Barley	(*Hordeum vulgare* L.)	Colonial	93	79
Bean	(*Phaseolus vulgaris* L.)	Contender	14	
Clover	(*Trifolium repens* L.)	Crimson	4	387*
Clover	(*Trifolium repens* L.)	Ladino	3	156
Clover	(*Trifolium repens* L.)	Red	0	107
		White Dutch	20	51
Corn	(*Zea mays* L.)	N.C. 27	4,439**	539*
Corn	(*Zea mays* L.)	Dixie 17	3,459**	
Corn	(*Zea mays* L.)	Ioana	995*	421*
Cotton	(*Gossypium hirsutum* L.)	Coker 100-W	1	265*
Crabgrass	(*Digitaria sanguinalis* (L.) Scop.)	Common	1,336**	
Crotalaria	(*Crotalaria mucronata* Desv.)	Striped	6	23
Crotalaria	(*Crotalaria mucronata* Desv.)	Giant striata	0	31
Cucumber	(*Cucumis sativus* L.)	Model		45
Fescue	(*Festuca elatior* L.)	Kentucky 31	0	
Lambsquarter	(*Chenopodium album* L.)	Common	0	
Lespedeza	(*Lespedeza stipulacea* Maxim.)	Rowan	48	
Lespedeza	(*Lespedeza cuneata* (Dumont) G. Don)	Sericea	0	
Lettuce	(*Lactuca sativa* L.)	Big Boston	0	4
Millet	(*Setaria italica* (L.) Beauv.)	Starr	831*	5
Milo	(*Sorghum vulgare* Pers.)	Martin	534*	
Oats	(*Avena sativa* L.)	Arlington	8	
Oats	(*Avena sativa* L.)	Fulgrain	89	53
Oats	(*Avena sativa* L.)	Victorgrain	33	
Peanut	(*Arachis hypogaea* L.)	N.C. 2		69
Pepper	(*Capsicum frutescens* L.)	California Wonder		30
Potato	(*Colanum tuberosum* L.)	Irish Cobbler		178
Rye	(*Secale cereale* L.)	Abruzzi	237*	15
Soybean	(*Glycine max* (L.) Merr.)	Lee	453*	
Soybean	(*Glycine max* (L.) Merr.)	Ogden		72
Sorghum	(*Sorghum vulgare* Pers.)	Common	1,842**	24
Strawberry	(*Fragaria chiloensis* Duch. var. *ananassa* Bailey)	Albritton		8
Sudan grass	(*Sorghum sudanense* (Piper) Stapf)	Common	421*	437*
Sweet potato	(*Ipomoea batatas* (L.) Lam.)	Porto Rico		15
Tobacco	(*Nicotiana tabacum* L.)	D.B. 101		571*
Tobacco	(*Nicotiana tabacum* L.)	Bottom Special		325*
Tobacco	(*Nicotiana tabacum* L.)	Bel. 6-32		511*
Tobacco	(*Nicotiana tabacum* L.)	Bel. 6-33		455*
Tomato	(*Lycopersicon esculentum* Mill.)	Rutgers	5	
Vetch	(*Vicia villosa* Roth)	Common hairy	0	164
Watermelon	(*Citrullus vulgaris* Schrad.)	Congo	2	
Wheat	(*Triticum aestivum* L.)	Atlas 66	105	

[a] Duration of test was 2 months, using 5 replicates of 6 in. clay pots for each plant species or variety.
[b] * = suitable host (more than 200 but less than 1000 nematodes per pot); ** = very suitable host (more than 1000 nematodes per pot).

each container, forming a ring about 9 in. in diameter. Four replications of each of the 3 soil type treatments were left fallow. All the containers were placed on a bench in the greenhouse with temperatures ranging from 70 to 80°F.

An additional container of Norfolk sandy loam was planted with corn to determine the best dates for sampling the main experiment. Soil and root samples taken from it 4 months after the beginning of the experiment indicated relatively high nematode populations 3 and 4 in. from the central core of infestation. At this time, soil and root samples were taken from the containers in the experiment. Just before being sampled with a soil tube 1 in. in diameter, the soil in the containers was moistened by frequent light applications of water to enable the removal of intact columns of soil. The surface area of each container was arbitrarily divided into 3 sectors, and soil samples were taken from each sector at positions 1–5 in. from the edge of the central column of original infestation. Each soil sample taken from the fallow soil was placed in a 10-qt. pail of water and mixed for several seconds. The suspension was poured on a 325-mesh screen, and the residue on the sieve was placed in a Baermann funnel to separate the nematodes from the debris. Each sample taken from the tubs containing corn was placed in a 10-qt. pail of water and agitated to free any root fragments from the soil. The contents of the pail were poured onto an 80-mesh sieve nested in a 325-mesh sieve. Root fragments collected on the 80-mesh screen were cut into smaller pieces and assayed for nematodes as previously described. Nema-

TABLE 2.—*Plant species and varieties tested for suitability as hosts to one species of root-lesion nematode, Pratylenchus brachyurus, in a 5-month test*

Name	Plants tested	Variety	Mean number of nematodes per pot[a]
Corn	(*Zea mays* L.)	N.C. 27	21,382
Soybean	(*Glycine max* (L.) Merr.)	Lee	4,590
Peanut	(*Arachis hypogaea* L.)	N.C. 2	3,859
Watermelon	(*Citrullus vulgaris* Schrad.)	Congo	1,384
Milo	(*Sorghum vulgare* Pers.)	Martin	1,200
Cotton	(*Gossypium hirsutum* L.)	Coker 100-W	558
Crabgrass	(*Digitaria sanguinalis* (L.) Scop.)	Common	246
Wheat	(*Triticum aestivum* L.)	Atlas 66	229
Oats	(*Avena sativa* L.)	Arlington	64
Crotalaria	(*Crotalaria mucronata* Desv.)	Striped	2

[a] Mean number of nematodes based on 5 replicates of 6-in. clay pots for each plant species or variety.

todes collected on the 325-mesh screen were separated from the soil with a Baermann funnel.

RESULTS.—*Host-range studies.*—The plants used in the host-range tests for *P. brachyurus* and *P. zeae* were arbitrarily divided into 3 groups, based on relative suitability as indicated by population increase or decrease during the 2-month test period. Suitability categories are indicated in Table 1. All plants tested with a particular species of nematode were classed in host favorability as a unit. Plants were not all tested simultaneously, but were tested in groups over a period under comparable greenhouse conditions.

Of the plants inoculated with *P. zeae*, corn (Varieties N.C. 27 and Dixie 17), sorghum, and crabgrass were very favorable for reproduction of the nematodes (Table 1). All plants classified as favorable for reproduction of *P. zeae*, except soybeans, are members of the family Gramineae. Results of host-range studies with *P. brachyurus* for tests of 2 months duration are also shown in Table 1. The plants classified as favorable for reproduction of this species include varieties of tobacco, corn, sudan grass, clover, and cotton.

Reproduction of *P. brachyurus* on host plants allowed to grow 5 months is presented in Table 2. Highest populations were found on corn (Variety N.C. 27). Other crops supporting high populations were soybean, peanut, watermelon, and milo. Lowest populations were found on crotalaria.

Infection and reproduction of P. brachyurus in relation to soil type.—Data on the numbers of nematodes found in 5 g of roots (Table 3) indicate that nematode populations on strawberry plants were greater in Norfolk sandy loam or Portsmouth loam than in the sand and Cecil clay, but the population difference between the Norfolk sandy loam and the Portsmouth loam was not significant. Nematode populations compared on the basis of whole root systems indicate the same over-all relationship, except that reproduction on plants was less in Portsmouth loam than in Norfolk sandy loam. No significant difference in population was found between plants grown in Portsmouth loam and either the sand or Cecil clay.

Nematode populations in 5-g root samples of cotton were significantly higher from Norfolk sandy loam than from any other soil type. No significant nematode population differences were found among plants grown in sand, Portsmouth soil, and Cecil soil. On the basis of whole root systems, populations on cotton plants were much higher from Norfolk sandy loam than any of the other soils. Nematode populations were higher on plants grown in Portsmouth loam than on plants grown in Cecil clay or sand.

The fumigants tested resulted in no significant differences in populations.

Migration of P. zeae as influenced by soil type and presence of host plants.—Four months after the start of the test, soil samples from non-seeded containers taken 1 in. from the edge of the original infestation yielded no nematodes. However, population means from samples taken at the center of each container of 4 replicates were 154 in Norfolk sandy loam, 197 in Portsmouth loam, and 12 in Cecil clay. Fig. 1 shows the numbers and positions of nematodes at this time in the containers seeded with corn. Samples taken from the Norfolk sandy loam at positions away from the center revealed that nematodes were located 5 in. from the center in 3 of the 4 containers. In the Portsmouth loam containers, nematodes were detected 3 in. from the center core, but were absent at a 5-in. distance in all containers except for one sector in one replicate. Relatively large numbers of nematodes were

TABLE 3.—*Mean[a] numbers of Pratylenchus brachyurus found in strawberry and cotton plants grown in 4 soil types*

Soil type	Number of nematodes			
	Strawberry		Cotton	
	5-g sample[b]	Total roots[c]	5-g sample[b]	Total roots[c]
Sand	86	174	315	823
Norfolk sandy loam	1964	2624	2107	14,442
Portsmouth loam	1150	1187	799	4,724
Cecil clay loam	258	666	200	825
LSD 5%	888	1109	623	3,471
LSD 1%	1209	1509	848	4,724

[a] Mean of 8 replicates (4 replicates of each of two soil fumigant treatments).

[b] Mean number of nematodes based on a 5-g sample of roots.

[c] Mean number of nematodes based on total weight of roots as determined from 5-g subsamples.

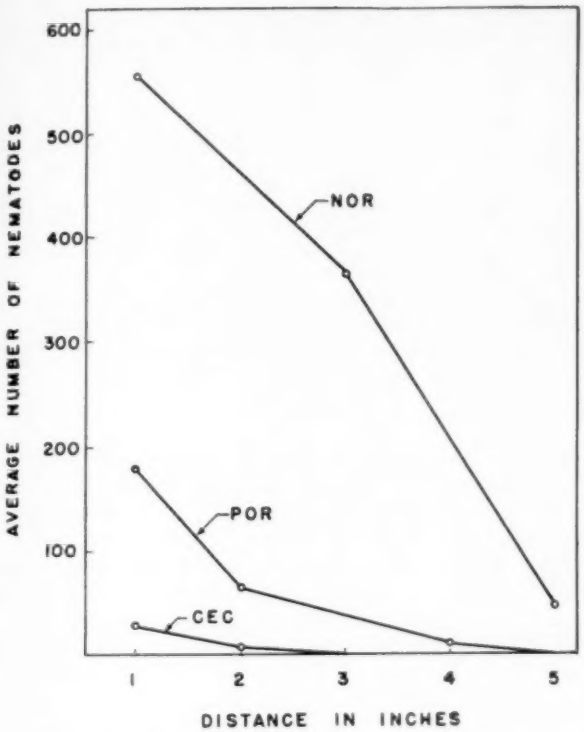

Fig. 1. Comparisons of horizontal movement of *Pratylenchus zeae* in the presence of corn plants in 3 soil types: Norfolk sandy loam (NOR), Portsmouth loam (POR), and Cecil clay loam (CEC). (Note: High count from one aberrant sector has been omitted from this presentation of the migration in the Portsmouth soil).

located at this area. In Cecil clay loam, nematodes were found at positions 2 in. from the central core of infestation. No nematodes were recovered 3 or 5 in. from the point of inoculation.

Discussion.—These experiments show the influence of certain ecological factors on the biology of 2 species of lesion nematodes. The host-range study based on population increase provides information on the relative suitability of the various host plants for reproduction of each of the nematode species. It indicates that not all plants are equally suitable for reproduction by these nematodes. Though this study has not provided information on the relative susceptibility of various crops to root decay, often associated with root-lesion nematode activity, it does provide information on the potential population that may develop on certain crop plants. Graham (2), in studying the relationship of *P. zeae* and *P. brachyurus* to brown root rot of tobacco, compared the relative susceptibility to these nematodes of some 20 cultivated and wild plants. However, for inoculum, he used nematode-infested soil that apparently contained undetermined numbers of both species of nematodes.

It was apparent from the soil-type study that Norfolk sandy loam provides a more suitable environment for root-lesion nematode activity than do Portsmouth loam, Cecil clay loam, or sand. Soil factors responsible for the population increase and survival in Norfolk sandy loam are not fully understood. Considerably lower populations of nematodes found on plants grown in Portsmouth loam and Cecil clay loam may reflect the indirect effect on nematode reproduction of soil aeration, pore space, particle size, and nematode motility.

It is apparent that the presence of plant roots is conducive to nematode migration, since little or no migration occurred in their absence. Since this study extended over a relatively long period, the observed spread was probably partly due to migration of nematodes from the original inoculum and partly due to migration of the progeny of these nematodes after reproduction in the corn roots. Even so, the maximum spread observed had an average rate of only 1¼ in. per month.

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THE EFFECT OF THE POLYENE ANTIBIOTIC FUNGICHROMIN ON THE FREE AND COMBINED AMINO ACIDS OF VERTICILLIUM ALBO-ATRUM[1]

J. P. Ross[2]

SUMMARY

Mycelium of *Verticillium albo-atrum* exposed to 10 and 30 ppm fungichromin lost all soluble amino acids. The composition of the bulk protein of the mycelium was altered significantly with respect to glutamic acid, threonine, alanine, lysine, arginine, and phenylalanine. At 2 ppm fungichromin, fungus growth was not reduced, nor were there major changes in the per cent composition of the protein, or excessive losses of soluble amino acids into the medium; however, there was a marked change in the relative composition of the hyphal free amino acid pool. The leucines, arginine, lysine, and especially proline were markedly increased. The total soluble nitrogen of the fungus was 3 times as great in cultures exposed to 2 ppm fungichromin as in cultures grown in the absence of fungichromin.

The metabolic action of certain antifungal polyene antibiotics has been the subject of research by Lampen, Morgan, and Slocum (2), at the Squibb Institute. From their results it was postulated that the effect of nystatin on the metabolism of various substrates is closely related to processes inhibiting the growth of cells. The effect of these antibiotics on nitrogen metabolism was apparently not investigated by this group. The effect of fungichromin[3] on the amino acid metabolism of *Verticillium albo-atrum* Reinke & Berth. is herein reported.

METHODS AND MATERIAL.—*V. albo-atrum* (peppermint isolate) was cultured in Coon's synthetic liquid medium (1.23 g $MgSO_4$, 2.72 g KH_2PO_4, 2.02 g KNO_3, 7.2 g maltose, 1000 cc distilled H_2O) (7). Culture vessels were 200-ml Erlenmeyer flasks, each containing 66 ml of medium. Each flask was seeded with 2 drops of a concentrated spore suspension, and the cultures were incubated on a reciprocating shaker at 21–22°C. After 6 days, sterile fungichromin was added to give final concentrations of 2, 10, and 30 ppm in the 3 flasks. Three control flasks received no antibiotic.

The hyphae were harvested 4 days after the antibiotic was added, and the culture liquids were concentrated by evaporation at room temperature and saved for analysis. After the hyphal fresh weights were obtained, the fungus was extracted with 70% ethanol. These extracts were quantitatively analyzed for amino acids by 2-directional paper chromatography, as described by Thompson, Zacharius, and Steward (6) and modified by Pollard (5). The first solvent was phenol saturated with water adjusted to pH 5.5; the second was *N*-butanol, glacial acetic acid, and water (9:1:2.9). The protein composition of the fungus hyphae was determined from the insoluble residue remaining after the alcoholic extraction. The residue was hydrolyzed in 6*N* HCl and centrifuged, and the supernatant was evaporated to dryness. After water had been added and evaporated 5 times, to drive off most of the hydrogen chloride, the hydrolyzate was neutralized with NaOH. The neutralized hydrolyzate was analyzed quantitatively for amino acids by paper-partition chromatography as outlined above. Before the culture media concentrates were analyzed, the solutions were passed through small cation exchange columns of Dowex-50 resin, 4% cross-linked, in the hydrogen form, to remove substances that interfere with amino acid movement on paper. The amino acids were removed from the columns by eluting with 0.15*N* NH_4OH and then 2*N* NH_4OH. The amino acid analyses of the culture media were qualitative.

RESULTS.—Fungichromin at 2 ppm did not inhibit the growth of the fungus, but 10 and 30 ppm inhibited growth considerably (Table 1). Preliminary experiments determined that 0.5 ppm fungichromin inhibited conidial germination of *V. albo-atrum*. In the experiment described, the mass of mycelium that had grown before addition of the antibiotic was undoubtedly large enough to absorb 2 ppm of the antibiotic without subsequent reduction in growth, whereas the higher concentrations (10 and 30 ppm) were beyond the threshold of tolerance, and little if any

TABLE 1.—*Growth and nitrogen distribution of 10-day-old Verticillium albo-atrum cultures 4 days after addition of various concentrations of fungichromin*[a]

Concentration (ppm)	Av. fresh weight (mg)	Total μg of N in alcohol-insoluble fraction, per mg fresh weight	Total μg of N in alcohol-soluble fraction, per mg fresh weight
0	129	5.09	0.25
2	183	5.75	0.73[b]
10	33	5.11	0.0
30	29	5.19[b]	0.0
LSD 1%	108		
LSD 5%	71		

[a]Average of 3 replicates.
[b]Average of 2 replicates.

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Portion of a thesis submitted to the Graduate Faculty of Cornell University in partial fulfillment of the requirements for the Ph.D. degree. The author is indebted to Dr. F. C. Steward for providing facilities for the chromatographic analyses.

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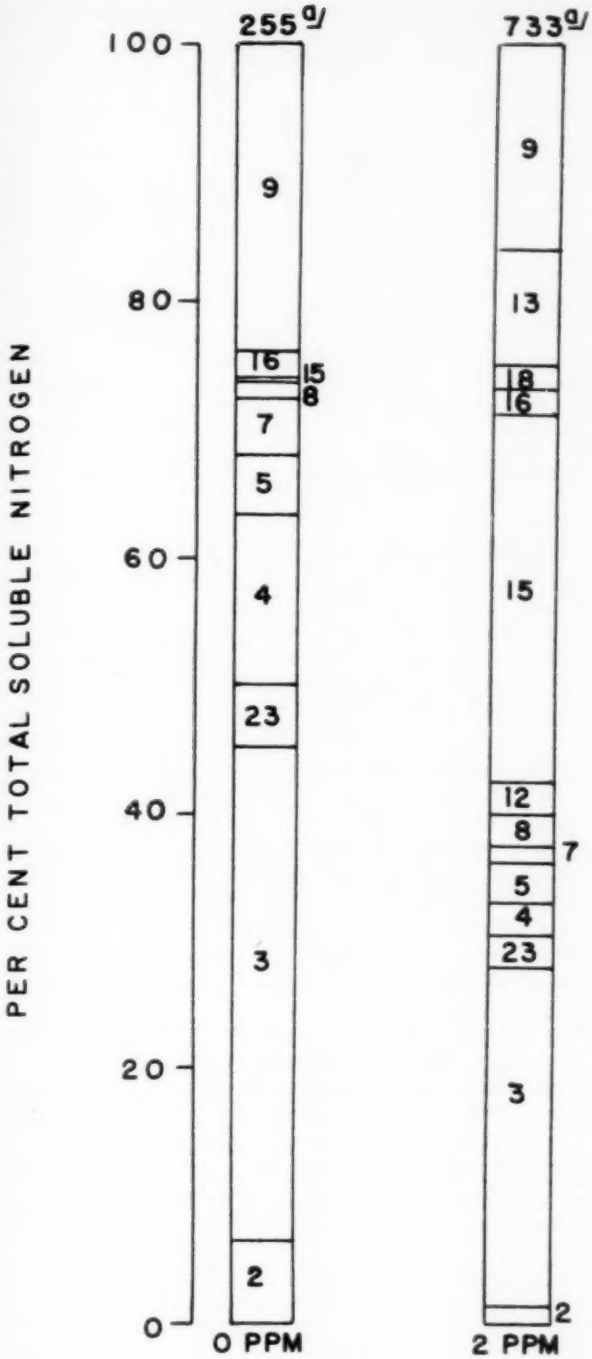
[3]Product of Merck and Co., Inc., Chem. Div., Rahway, N. J.



*Total micrograms of soluble nitrogen.

Fig. 1. The relative composition of the soluble nitrogen fraction of 10-day-old *V. albo-atrum* cultures 4 days after addition of 2 ppm fungichromin. (The nitrogen of each amino acid is expressed as a percentage of the total nitrogen soluble in alcohol as determined by reaction with ninhydrin.) Key to the amino acids: 2, aspartic acid; 3, glutamic acid; 4, serine; 5, glycine; 7, threonine; 8, alanine; 9, glutamine; 12, lysine; 13, arginine; 15, proline; 16, valine; 18, leucine and/or isoleucine; 23, gamma amino butyric acid.

growth ensued.

The nitrogen present in each free amino acid in the hyphal extract is presented as the percentage of the total amino acid nitrogen (Fig. 1). Amino acids were detected only in extracts from cultures exposed to 2 ppm fungichromin, and from control cultures. Two ppm of the antibiotic had a pronounced effect on both the total amino nitrogen and the per cent composition of the free amino acid pool. The large increase in proline was apparently at the expense of most of the other amino acids. Also noteworthy was the appearance of substantial quantities of the leucines and of the basic amino acids, arginine and lysine.

The nitrogen of each amino acid from the protein hydrolyzates is represented as the percentage of total protein amino acid nitrogen (Table 2). There was no difference between the per cent composition of the protein of the untreated cultures and that of those exposed to 2 ppm fungichromin, except for valine. The alterations in the protein composition that resulted from exposing the fungus to 10 and 30 ppm was significant for glutamic acid, threonine, alanine, lysine, arginine, and phenylalanine. As indicated by Block and Bolling (1), one of the main sources of error in analyzing protein is the loss of amino acids during hydrolysis. In all probability some tyrosine, phenylalanine, arginine and histidine was lost during the hydrolysis of protein in the present investigation. Tryptophane and any amides, if present in the protein, would probably be totally destroyed.

The nitrogen present in both the soluble and insoluble fractions was increased over that of the controls by the 2-ppm treatment (Table 1). Cultures treated with 10 and 30 ppm contained about the same amount of insoluble nitrogen as did the controls, but no soluble nitrogen.

Although the analyses of the culture fluids were only qualitative, the chromatograms showed quite distinctly that relatively small quantities of amino acids were present in both the medium receiving no fungichromin and that receiving 2 ppm (Table 3). In contrast, large quantities of amino acids were present in the medium of cultures treated with 10 and 30 ppm of antibiotic. Alanine and glutamic acid were consistently in the highest concentrations.

Discussion.—Both the significant alteration of the free amino acid pool and the 3-fold increase in total free amino acid nitrogen manifested by *V. albo-atrum* grown in a sublethal dose of the antibiotic indicate that the amino acid metabolism of the fungus is affected by the antibiotic. The exact point(s) at which fungichromin affects fungus metabolism cannot be determined from the present information. The increase in free amino acid nitrogen coincident with a slight increase in total protein nitrogen (at 2 ppm) is indicative of a net synthesis of organic nitrogen compounds.

The presence of large quantities of amino acids in the media of cultures exposed to 10 and 30 ppm fungichromin probably represents a loss of the soluble

TABLE 2.—*The per cent amino acid composition of the alcohol-insoluble protein of 10-day-old Verticillium albo-atrum cultures 4 days after the addition of various concentrations of fungichromin*

Treatment	Fungichromin concentration (ppm)	Amino acids[a,b]													
		2	3	4	5	7	8	11	12	13	15	16	18	19	21
1	0	6.7	8.5	8.0	6.9	5.8	8.9	1.9	5.7	8.4	9.7	6.0	13.0	6.2	4.3
2	2	8.5	9.4	7.7	7.9	5.7	8.4	0.8	5.3	6.9	10.9	4.9	12.2	8.3	3.4
3	10	5.2	5.1	13.5	8.6	8.3	10.0	tr	2.8	4.2	tr	6.9	16.7	12.7	3.0
4	30	3.4	5.1	10.6	6.5	6.2	7.1	tr	2.8	4.0	16.2	6.6	16.3	11.7	3.5
Statistical comparisons of treatments[c]		2-4**	1-3* 1-4* 2-3** 2-4**	NS	NS	1-3** 2-3**	1-4** 3-4**	[d]	1-3** 1-4** 2-3* 2-4*	1-3** 1-4**	[d]	1-2* 2-3** 2-4**	NS	1-3** 1-4** 2-3* 2-4*	NS

[a]Methionine sulfoxide present as trace in all treatments.

[b]Figures entered in the table represent amino acid nitrogen as per cent of total protein nitrogen as determined by reaction with ninhydrin. Key to amino acids: 2, aspartic acid; 3, glutamic acid; 4, serine; 5, glycine; 7, threonine; 8, alanine; 11, histidine; 12, lysine; 13, arginine; 15, proline; 16, valine; 18, leucine and/or isoleucine; 19, phenylalanine; 21, tyrosine.

[c]* and ** respectively denote significant and highly significant differences between the 2 treatments designated.

[d]No analysis computed.

amino acids that were present in the mycelium before treatment. In contrast, asparagine and tyrosine were detected in the culture media at the 2 higher concentrations of the antibiotic, but not among the free amino acids of the hyphae of untreated and 2-ppm-treated cultures. Hence, the presence of asparagine and tyrosine might also indicate some protein breakdown. Tyrosine was detected in the protein hydrolyzate; asparagine would have been destroyed during the acid hydrolysis. An increase in both protein and free amino acid nitrogen was observed in cultures treated with 2 ppm fungichromin. If a similar increase occurred in cultures treated with 10 and 30 ppm prior to the loss of soluble nitrogen compounds, this might account for the large increase of amino acids in the culture medium without any over-all reduction of the total protein nitrogen.

Morton and Broadbent (4) found that 60–85% of the extracellular nitrogen of the liquid cultures of *Scopulariopsis brevicaulis* Bainer was present as peptides, and only 10–15% as free amino acids. Although the extracellular amino acids of *V. albo-atrum* were only qualitatively analyzed, the concentration of amino acids in the medium of cultures treated with 10 or 30 ppm fungichromin seemed greater than the concentration of intracellular and extracellular free amino acids of untreated cultures. This suggested that some of the extracellular amino acids may have been derived from peptide breakdown. However, when samples of hydrolyzed and nonhydrolyzed medium concentrate from an untreated culture were compared chromatographically, no significant difference in amino acid content was noted. Hence, it does not seem tenable that the large amounts of amino acids in the medium arose from extracellular peptides unless these were lost during passage of the medium of the untreated cultures through the cation exchange column.

Morton and Broadbent (4) found that alanine, glutamic, aspartic, and gamma amino butyric were the principal amino acids present in the medium during early growth of *S. brevicaulis*. The results with *V. albo-atrum* are similar for alanine and glutamic acid, but differ for gamma amino butyric and aspartic acid.

Lampen, Morgan, and Slocum (2) reported that other polyene antibiotics, such as nystatin and amphotericidin, inhibit endogenous respiration and anaerobic utilization of carbohydrates by fungi. Amino acid metabolism is related to the Krebs' cycle, glycolysis, and the terminal oxidase systems through transamination reactions (3). Therefore, the observed effect of fungichromin on amino acid metabolism may be either

TABLE 3.—*Amino acids present in the media of 10-day-old Verticillium albo-atrum cultures 4 days after the addition of various concentrations of fungichromin*

Concentration (ppm)	Amino acids[a,b]														
	2	3	4	5	6	7	8	9	12	13	15	16	18	21	23
0	tr[c]	+	tr	tr	0	0	+	0	tr	0	0	tr	0	0	0
2	tr	++	+	+	0	+	++	0	tr	0	0	+	0	0	+
10	++	++++	+++	+++	++	++	++++	++	tr	+	+	++	+	tr	+++
30	++	++++	+++	++	++	++	++++	++	tr	0	++	+++	++	tr	+++

[a]Entries in table represent visual comparisons of color intensities of spots on chromatograms, as follows: 0=not detected, tr=barely visible, +=light spot, ++=moderate spot, +++=heavy spot, ++++=very heavy spot.

[b]Key to the amino acids: 2, aspartic acid; 3, glutamic acid; 4, serine; 5, glycine; 6, asparagine; 7, threonine; 8, alanine; 9, glutamine; 11, histidine; 12, lysine; 13, arginine; 15, proline; 16, valine; 18, leucine and/or isoleucine; 21, tyrosine; 23, gamma amino butyric acid.

[c]Trace.

a direct or indirect manifestation of action at any one of several points.

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COMPARATIVE POPULATION TRENDS OF XANTHOMONAS PHASEOLI IN SUSCEPTIBLE, FIELD TOLERANT AND RESISTANT HOSTS[1]

Albert L. Scharen[2]

SUMMARY

Population trends of *Xanthomonas phaseoli* (E. F. Smith) Dowson in the leaf tissues of resistant, field tolerant, and susceptible bean species were determined. Large numbers of bacterial cells used for inoculum, using the water-soaking inoculation technique, caused symptoms and subsequent destruction of the tissues of the three species. Lower numbers of bacteria used in inoculum and using the water-soaking inoculation technique resulted, after a longer incubation period, in disease symptoms and death of the field tolerant and susceptible species, but no symptoms in the resistant species. Relatively large numbers of bacteria persisted in resistant tissues without production of symptoms. Other inoculation techniques tested indicated that symptoms could be produced in the resistant species only by water-soaking with large numbers of bacteria. All inoculation techniques tested resulted in production of typical symptoms by the pathogen in the susceptible and field tolerant species. The evidence presented suggests the possibility of a host reaction that may temporarily reduce the bacterial population in all cases. In the case of the resistant host, further bacterial multiplication may be precluded.

Common blight of beans is a disease that causes material losses of yield and quality in many of the principal bean-producing areas. The malady and its incitant, *Xanthomonas phaseoli* (E. F. Smith) Dowson, have consequently been the subject of many studies (6). Several attempts to breed resistant varieties have been unsuccessful. It has been reported that the Tepary bean, *Phaseolus acutifolius* A. Gray var. *latifolius* Freeman, is highly resistant to common blight (3), and the variety Scarlet Runner, *P. coccineus* L., exhibits field tolerance. Attempts have been made to use these as resistant parents and the variety Great Northern, *P. vulgaris* L., as the parent possessing desirable horticultural characteristics.

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Investigations have shown that pathogenic bacteria may persist and multiply appreciably in the tissues of resistant and immune varieties (1, 2, 4). The fact that large numbers of individuals are produced in these resistant or immune varieties suggested that some insight into the constitution of disease resistance and the ability of a resistant variety to maintain itself over a period might be gained by a study of the population trends of *X. phaseoli* in the leaf tissues of Great Northern, Scarlet Runner, and Tepary beans.

MATERIALS AND METHODS.—An isolate of *X. phaseoli* was obtained from naturally infected beans grown in western Nebraska. The plant species employed throughout the experiments were *P. vulgaris*, var. Great Northern; *P. coccineus*, var. Scarlet Runner; and the Tepary bean, *P. acutifolius* var. *latifolius*. Seeds of these species were germinated in a rag doll and then planted, 1 per 5-in. pot. Before inoculation, the plants were grown in a greenhouse at temperatures ordinarily fluctuating from 70° to 95°F. After inoculation, they were transferred to an air-conditioned greenhouse maintained at 65–75°F.

Inoculum was prepared from 24-hour cultures of

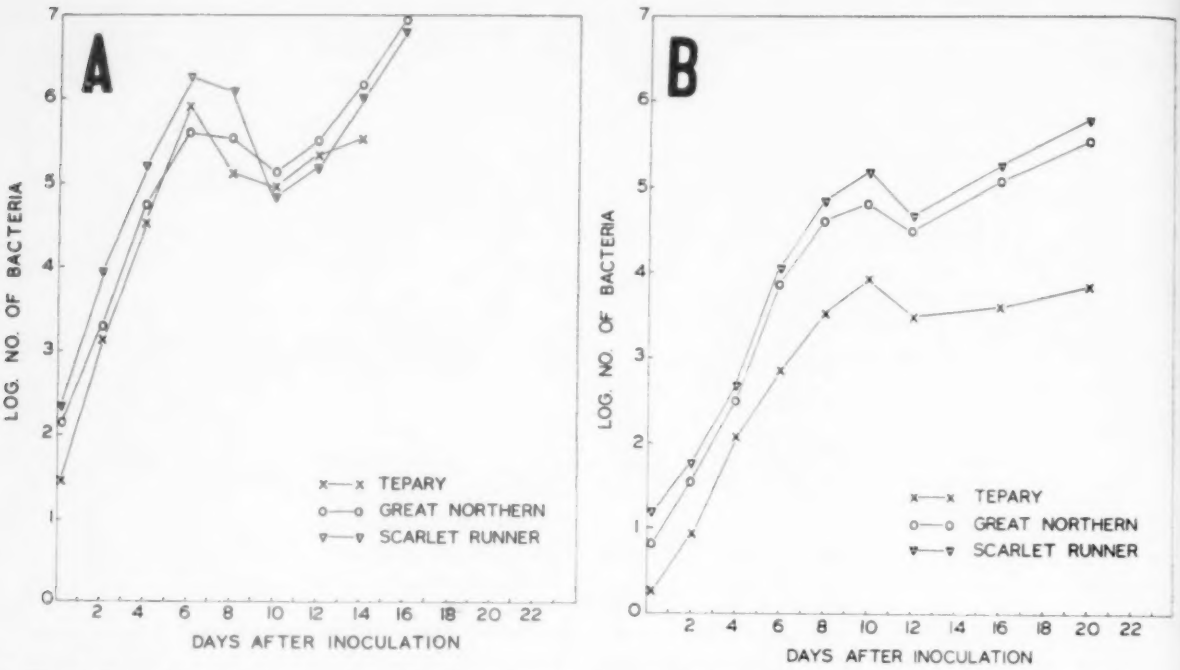

Fig. 1. Bacterial population trends in the 3 bean species after inoculation with *X. phaseoli*. **A)** Arithmetic mean values of 4 experiments with inoculum containing 10^6 bacterial cells per ml. **B)** Arithmetic mean values of 4 experiments with inoculum containing 2×10^4 bacterial cells per ml.

X. phaseoli grown on Burkholder's medium.[3] The bacteria were washed from the medium with distilled water and the resulting suspension was adjusted to a standardized turbidity.

Further dilutions were made to obtain the desired concentration of bacterial cells for any particular experiment. The number of viable cells was always estimated by plate counts.

Inoculation was accomplished by water-soaking the leaves with a bacterial suspension employing an atomizer designed with an electrically driven blower or a de Vilbiss No. 127 atomizer attached to an air line having 15 lb. pressure. As the liquid was forced into the leaf through the stomata, that part of the leaf in which the air in the intercellular spaces was replaced by liquid became visibly water-soaked. In most of the inoculations the total leaf area was water-soaked, but in those instances where leaf size was great, as with Scarlet Runner, only a portion of the leaf about 1 in. wide and 2 in. long on both sides of the midrib was water-soaked. After evidence of water-soaking disappeared, the leaves were rinsed with distilled water to remove bacterial cells on the leaf surface.

In each test 3 trifoliate leaves of similar position and age were selected on each of the plants to be utilized. The bacterial population was estimated by taking tissue samples from 3 leaflets, each was at a different position, i.e., high, middle, or low, and each from a different plant. This method of sampling was designed to compensate for differences between plants and differences in age of leaves on any one plant. The tissue samples were taken by cutting out disks with a 4-mm cork borer. Eighteen disks, 6 from each of the 3 leaflets, were thoroughly macerated in 10 ml of sterile distilled water and allowed to stand 1 hour, with intermittent shaking. Eight serial dilutions, descending at a multiple rate of 10, were made in sterile water, and 1 ml of each was added to 1 Petri dish. Burkholder's agar was then added and thoroughly mixed. Colony counts were made after incubation for 6–7 days at 75°F. The first leaf samples were always taken within 1 hour of inoculation except in 1 case, as indicated below. Sampling usually continued at 2-day intervals for 17–25 days, unless the leaves withered so as to preclude further determinations.

EXPERIMENTAL RESULTS.—Four experiments were made with inoculum containing 10^6 viable cells per ml. In each experiment, 6 plants of each species were selected, and 3 corresponding leaves on each plant were inoculated.

Results of the plate counts showed that bacteria multiplied rapidly within the tissues in all 3 species until 5–6 days after inoculation. In most cases, bacterial numbers declined at this time. Subsequent increases in numbers of bacteria in the tissues were invariably correlated with the appearance of symptoms. The Tepary leaves developed unusual symptoms. The midrib and veins became bright yellow, whereas the interveinal areas were dark green though necrotic.

[3]Composed of: extract of 300 g potatoes; peptone, 5 g; sodium phosphate, 2 g; sodium chloride, 2 g; sodium citrate, 1 g; dextrose, 6 g; agar, 17 g; and asparagine, 1 g in 1 liter of water.

This color pattern was ordinarily retained until the leaves had dried. Leaves of Great Northern and Scarlet Runner developed necrotic lesions surrounded by lemon-yellow borders typical of this disease in the field.

The graph of the arithmetic mean values of the 4 experiments (Fig. 1-A) shows that no appreciable differences appeared between bacterial populations in the three host species until 5–6 days after inoculation. An erratic decline in population was then apparent, followed by a rapid increase in bacteria and death of the tissues. It is striking that Tepary, the presumably resistant species, was killed sooner and by a lower population of bacteria than the two susceptible species, Great Northern and Scarlet Runner.

Since the inoculum concentration used in the preceding experiments was relatively high and the results obtained were similar in both resistant and susceptible species, a second series of experiments was made. This series employed a lower concentration of inoculum, one more likely to occur under natural conditions. The purpose was to determine whether the resistance of Tepary might have been overcome by the relatively large numbers of the pathogen.

Four experiments were conducted with inoculum containing about 2×10^4 viable bacteria cells per ml. In each of these experiments 3 plants of each bean species were selected, the leaves to be sampled were tagged, and inoculations were made with the de Vilbiss atomizer.

The results (Fig. 1-B) showed a greater uniformity than was obtained in the foregoing experiments with a more concentrated inoculum. Fewer bacteria entered the leaf tissues during inoculation, and multiplication of the pathogen in the tissues proceeded more slowly. Fewer numbers of the pathogen were found in Tepary than in the other two host species at all stages of the experiments. The population of the pathogen reached the first peak about 10 days after inoculation. No marked symptoms had yet developed in any of the plants; only a slight yellowing in Great Northern and Scarlet Runner. In Tepary, the bacterial population within the tissues decreased sharply, then increased very slowly. Symptoms did not appear, and inoculated leaves remained on the plants as long as uninoculated leaves. Inoculated leaves of Great Northern and Scarlet Runner withered about 3 weeks after inoculation.

OTHER INOCULATION TECHNIQUES.—During preliminary experiments Tepary beans showed external symptoms of common blight only after inoculation by visibly water-soaking the leaf tissues with suspensions containing 10^6 bacteria or more. A series of experiments used 2 methods of inoculation other than water-soaking in order to determine and compare the reactions of the 3 host species.

Spraying.—One plant of each bean species was sprayed with a fine mist of inoculum containing 2×10^7 bacterial cells per ml until droplets of inoculum covered the surface of all leaves. No visible water-soaking was induced. The plants were then placed in moist chambers at 22°C. At 48 hours, they were removed to a greenhouse at 75–90°F.

Eight days later small water-soaked spots had appeared on the leaves of Great Northern and Scarlet Runner, and at 12–15 days large necrotic lesions, surrounded by yellow chlorotic bands, had developed. No symptoms had appeared on Tepary. At 3 weeks, many leaves of Great Northern and Scarlet Runner had withered, and their appearance was typical of bean blight symptoms in the field. Tepary appeared normal throughout the experiment.

Results were comparable when the test was subsequently repeated 3 times.

Needle puncture.—Six leaflets on 1 plant of each host species were each punctured 50 times with a sterile dissecting needle. The punctures were confined to a circular pattern about 1 in. in diameter near the center of the leaf and on each side of the midrib. Immediately after, the leaves were immersed in a suspension of *X. phaseoli* containing about 10^7 cells per ml. The plants were then placed in a greenhouse at 75–90°F.

After 8 days, large chlorotic and necrotic areas had developed on the leaves of Great Northern and Scarlet Runner. Only very small dry borders appeared around the needle punctures on Tepary. Fifteen days after inoculation, extensive areas of necrosis, surrounded by yellow borders, were apparent on all inoculated leaves of Great Northern and Scarlet Runner. At 30 days, all inoculated leaves of the two susceptible species had withered, but no symptoms were detected in any of the Tepary leaves.

This test was repeated twice, with comparable results.

DISCUSSION.—It has been known for some time that certain pathogenic bacteria have the ability to persist and multiply in the tissues of resistant and immune host plants (1). The results of the current studies, however, differ somewhat from those previously reported. A normal geometric progression of bacterial multiplication, which was to be expected, did not occur in the tissues investigated. The population of *Xanthomonas phaseoli* in both susceptible and resistant leaf tissues increased steadily at about equal rates for several days after inoculation. The numbers of the pathogen then declined temporarily, and then increased to a maximum.

Weindling (5), from work with artificial inoculation of *Xanthomonas malvacearum* in cotton, said that the larger the initial number of bacteria introduced per unit volume of intercellular space, the shorter the period necessary for incubation and the greater the chances for further spread through the tissues. This statement seems to fit the results presented in this paper. When a large number of bacteria were introduced into the leaves of Tepary, the pathogen appeared capable of overcoming the resistant reaction of the host, proceeding to multiply, and ultimately destroying the tissues. However, a relatively smaller initial number of cells does not seem to be able to surmount the resistant reaction, and the relationship is turned in favor of the host. Although large num-

bers of bacteria remain alive in the leaves of the resistant host, no symptoms appear, and further spread of the pathogen in the plant does not occur.

Further experimental support for this contention accrued as a result of the studies with inoculation procedures other than water-soaking of leaves. With the same inoculum concentration, the alternative methods of inoculation employed probably do not introduce as many bacterial cells into the intercellular spaces as does water-soaking. The longer period ensuing before development of symptoms on the susceptible species, and the total absence of symptoms on the resistant species, appears to corroborate the hypothesis. The knowledge that highly concentrated inoculum accompanied by water-soaking is necessary for infecting leaves of Tepary may also explain the absence of bacterial blight on this species under natural conditions.

The available evidence seems to indicate that the temporary decline in bacterial numbers within the tissues is due to a host reaction that is manifested only after considerable numbers of the pathogen are present. This hypothesis is in accord with that proposed by Allington and Chamberlain (1). The mechanism of this resistant reaction is unknown.

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NEW RACES FROM MIXTURES OF UREDIOSPORES OF VARIETIES OF PUCCINIA GRAMINIS[1]

G. H. Bridgmon and Roy D. Wilcoxson[2]

SUMMARY

This is the first report of genetic recombination between varieties of *Puccinia graminis* during the uredial stage of the fungus. Orange urediospores of *tritici* race 11 were mixed with red urediospores of *secalis* in the boots of barley plants, where the recombinations occurred. Three new races were isolated from Little Club wheat, which had been inoculated with urediospores from the barley plants. The new races each had red urediospores and attacked fewer of the differential varieties than did the *tritici* parent, but more than did the *secalis* parent.

Recent reports (1, 5, 6, 9, 10) of new races of rust arising by genetic exchange between different races of rust fungi during the uredial stage have been based on single varieties of the fungi concerned. Several workers (3, 4, 7) have studied intervarietal hybrids of *Puccinia graminis* Pers. obtained from barberry; and *P. graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Shear, Ball, Jacks., & Stakman appeared to hybridize readily with *P. graminis* Pers. var *secalis* (Eriks. & E. Henn.) Seym. The present work was done to determine whether urediospore germ-tube fusions between different varieties of *P. graminis* might permit recombination of genetic materials, forming new races.

Materials and methods.—Single-urediospore isolates were obtained of varieties *tritici* (race 11, with orange urediospores) and *secalis* (red urediospores). Before urediospores were mixed, stability was observed for several uredial generations of the infection types produced on the standard differential wheats (8) plus Lee, Bowie, Kenya Farmer, and Tetra Petkus rye.

Several days before the heads emerged, two separate mixtures of about equal quantities of urediospores of the 2 isolates were suspended in distilled water and immediately injected into the boots of 160 Kindred barley plants. Twenty days later the entire mass of urediospores from barley was suspended in oil (Mobisol 100) and sprayed onto seedlings of Little Club, Bowie, Kenya Farmer, Khapli, and Vernal wheats, and onto Kindred barley and Bond oats. All

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TABLE 1.—*Reactions[a] of differential varieties to P. graminis var. tritici race 11 and P. graminis var. secalis and to 3 new races obtained from mixing urediospores of these rust varieties*

Varieties	Parent races		New races		
	Orange *tritici*, race 11	Red *secalis* isolate	A	B	C
Little Club	4	0;	3=c	3	3
Marquis	4–	0	0;1[b]	2	3
Kota	4–	0;	0;	3	3
Reliance	4–	0;	0	3	3
Arnautka	3	0	0;1	0;1	X
Kubanka	4–	0;	0;	0;1	0;1
Einkorn	3+	X=	3	0;1	0;1
Acme	3+	0;	0	3	0;1
Tetra Petkus rye	0	4	0;1	1	1

[a]Infection types are as follows: O = immune, O; = flecks, 1 = very resistant, 2 = moderately resistant, 3 = moderately susceptible, 4 = very susceptible, X = heterogeneous; + and — indicated variations within each type; c = chlorosis. Infection types of the new races and *secalis* parent on Mindum, Spelmar, Vernal, Khapli, Bowie, Lee, and Kenya Farmer were resistant, like those on Kubanka. On these wheats the *tritici* parent caused susceptible infection types only on Mindum and Spelmar.

[b]Both infection types were present on all plants.

plants were examined for red uredia, which formed only on Little Club and barley. These uredia were increased on Little Club, and racial determinations were made on the differential varieties listed above. The work was done in the greenhouse in late winter and early spring to reduce the possibility of contamination from outside sources.

RESULTS.—Only parent races were recovered from the first mixture. From the second mixture, 3 new races of wheat stem rust were isolated from Little Club (the red uredia on barley all proved to be *P. graminis* var. *secalis*). Infection types and varieties that distinguished the new races from each other are listed in Table 1. The infection types of the 3 new races and of the *secalis* parent on Mindum, Spelmar, Vernal, Khapli, Lee, Bowie, and Kenya Farmer were similar to those on Kubanka. The *tritici* parent produced susceptible infection types on only Mindum and Spelmar of these wheats.

Each new race had red urediospores, as did the *secalis* parent, but was more pathogenic on several wheats than was *secalis*. New race A was weakly pathogenic on Little Club and only moderately pathogenic on Einkorn. New races B and C differed from new race A on several wheats, and differed from each other on Acme, Arnautka, and Marquis. New race B differed from race 173 only because the latter race causes infection type 4 on Kubanka. Spore color and pathogenicity of the new races were stable through 2 uredial generations, after which tests were discontinued.

DISCUSSION.—This is the first report of new races arising by recombination of genetic materials from 2 varieties of *P. graminis* during the uredial stage. Factors for urediospore color from *secalis* and for pathogenicity from *tritici* were recombined in the 3 new races. Several facts lead to the conclusion that the new races arose from recombination of materials from the 2 fungus varieties: 1) the new races had red urediospores; 2) they all originated in the same mixture; 3) the new races have not been described previously; 4) the new races attacked more of the differential varieties than did the *secalis* parent but fewer than the *tritici* parent, which has been characteristic of many hybrids of *tritici* and *secalis* from barberry (3, 4, 7); 5) the new races rather closely resembled races 36, 57, 67, 70, 71, 103, 104, 111, and 112, all of which have been described as hybrids of *tritici* and *secalis*.

Work of Wilcoxson, Tuite, and Tucker (11) and Hermansen (2) suggests that other intervarietal "hybrids" might also be formed when urediospores are mixed. However, one trial with a mixture of *tritici* and *P. graminis* Pers. var. *agrostis* (Eriks. & E. Henn.) Seym. did not yield new races. This type of work might also help clarify the origin of *P. graminis* Pers. var. *phlei-pratensis* (Eriks. & E. Henn.) Stakman & Piemeisel, which attacks timothy grass (*Phleum pratense* L.) but not barberry.

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PURPLE STAIN OF SOYBEAN SEEDS INCITED BY SEVERAL CERCOSPORA SPECIES[1]

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SUMMARY

Eighteen isolates of *Cercospora* representing 13 species isolated from 13 separate host species were inoculated into attached developing soybean pods. Ten of the 13 *Cercospora* species incited purple-stained soybean seeds indistinguishable from those produced by *C. kikuchii*, the purple seed stain fungus of soybean. The purple discoloration was found to be due to the presence of the fungi within the seed coats. The *Cercospora* isolates showed a correlation between the ability to incite purple stain of soybean seeds and the production of purple pigment in pure culture.

The purple seed stain disease of soybean, *Glycine max* (L.) Merrill, is caused by the fungus *Cercospora kikuchii* T. Matsu. & Tomoyasu. No other fungus species has been reported to incite purple stain of soybean seeds under natural conditions. Kilpatrick and Johnson[3] observed, however, that several isolates of *Cercospora* species, obtained from a number of different hosts, would discolor soybean seeds pink to purple in culture. They surface-sterilized mature soybean seeds, plated them on potato-dextrose agar in Petri dishes, and inoculated them with a cube of *Cercospora* inoculum. Most of the species tested produced a pink to purple discoloration of the soybean seeds. The results indicated that purple-staining of soybean seeds may be caused by species of *Cercospora* other than *C. kikuchii*. Since this observation was based on evidence obtained in culture on mature seeds, it was questioned whether results would be similar with inoculation of the developing host. The present experiment was conducted to determine if a number of *Cercospora* species would incite purple stain of soybean seeds when inoculated into the growing plant.

MATERIAL AND METHODS.—In preliminary experiments, a method of hypodermic injection of attached soybean pods with macerated mycelium of *C. kikuchii* was developed that produced typical purple-stained seeds. Pods were injected in the "filled" stage, since injection of younger pods in the "flat" stage usually destroyed the developing seeds. Injection was made at either the floral or stem end of the pod, with the needle tip just entering the cavity. The liquid was then forced throughout the pod until it was uniformly distributed, a process that could be observed through the pod walls. In this manner the seed was not injured by the needle. The amount of liquid injected was 0.05–0.10 ml, depending on the number of seeds per pod. Inoculated plants were allowed to mature with no further treatment.

Greenhouse-grown plants of the soybean variety Norchief were used in the experiment. The plants were grown in 8-in. pots of potting soil, 2 plants per pot. At inoculation, each plant bore 4–6 pods containing 1–3 seeds per pod.

Included in the study were 18 isolates of *Cercospora* —9 from leguminous and 9 from nonleguminous hosts. The 18 isolates consisted of 13 *Cercospora* species from 13 different host species. All but 3 of the isolates were secured by direct-spore transfer from infected host material. The 3 exceptions were the isolate of *C. capsici* (from W. W. Hare, Mississippi State University) and the 2 cultures of *C. kikuchii* (isolated from soybean seed coats).

The fungi were cultured in 25-ml lots of potato-dextrose broth contained in 125-ml Erlenmeyer flasks. Inoculum was prepared by filtering off the mycelial mats and macerating them in distilled water with a food blender. Inoculations were made with B-D Yale tuberculin syringes and 25-gauge needles. Two types of checks were used: pods inoculated with distilled water, and untreated plants.

EXPERIMENTAL RESULTS.—Two separate inoculation series were made in the experiment. The first inoculation series was carried out with 17 *Cercospora* isolates. All the pods on 2 plants were inoculated with each isolate. Inoculum concentration of each isolate consisted of two 14-day-old mycelial mats per 100 ml of distilled water. Plants were injected, and placed on a greenhouse bench and allowed to mature. Seed was harvested 16 days after inoculation. The results of examination are summarized in Table 1.

Ten of the 13 *Cercospora* species incited typical purple-stained soybean seeds (Fig. 1). The *Cercospora* species from leguminous hosts that produced purple stain included *C. kikuchii* from soybean seeds and stems (all 3 isolates), *C. medicaginis* from alfalfa leaflets, *C. zebrina* from red clover leaflets, and *C. canescens* from cowpea stems. Two species from leguminous hosts did not cause purple stain. They were *C. sojina*, the frogeye fungus from soybean leaflets, and *C. cruenta* from cowpea leaflets.

The *Cercospora* species from nonleguminous hosts that produced purple stain were *C. brachiata* from pigweed leaves, *C. capsici* from pepper leaves, *C. gossypina* from cotton leaves, *C. malayensis* from okra leaves, *C. ricinella* from castorbean leaves, and *C. sesami* from sesame leaves and stems. Neither isolate of *C. sorghi* from sorghum or Johnson grass leaves caused a purple-staining of the soybean seeds.

It was also observed that the walls of the pods con-

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[2]Pathologist, Crops Research Division, ARS, U.S.D.A., and Mississippi Agricultural Experiment Station, cooperating.

[3]Kilpatrick, R. A., and Howard W. Johnson. 1956. Purple stain of legume seeds caused by *Cercospora* species. Phytopathology 46: 201-204.

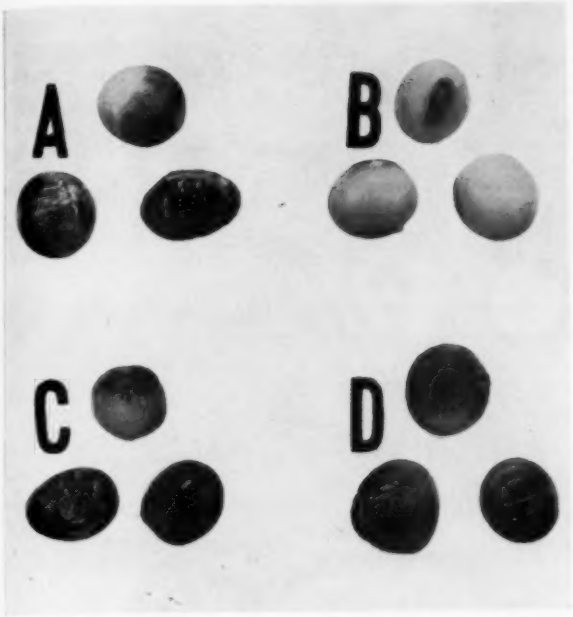

Fig. 1. Soybean seeds showing purple discoloration resulting from inoculation of pods with several *Cercospora* species. **A)** *C. kikuchii*; **B)** water-inoculation; **C)** *C. gossypina*; **D)** *C. brachiata*.

taining purple-stained seeds were discolored dark gray, similar to naturally infected pods. The nonpurple-staining isolates of *C. sojina*, *C. cruenta*, and *C. sorghi* produced occasional brown spots on many of the seeds, but no pod-wall discoloration. No discoloration of any type was noted in the seed coats or pod walls of the water-inoculated and no-treatment checks.

The second inoculation series was conducted with all 18 isolates at one-half the inoculum concentration of the first series, except for the nonpurpling isolates *C. sojina*, *C. cruenta*, and *C. sorghi*, which were used at the original concentration. Fungus mats were one week old when prepared as inoculum. The nonpurpling isolates, crimson clover isolate, and water check were inoculated into 4 plants each, while 2 per treatment were used for the remaining isolates. Eight plants were used for the no-treatment check. Seed was harvested 24 days after inoculation. The data from the second inoculation series are also in Table 1.

The results of the second inoculation series were similar to those obtained in the first series. Except for *C. sojina*, *C. cruenta*, and both isolates of *C. sorghi*, all the *Cercospora species*, including *C. zebrina* from crimson clover, produced purple-staining indistinguishable from that produced by *C. kikuchii*. It was noted, however, that the proportion of purple-stained seed was less in some cases than in the first inoculation series, but this was ascribed to the reduced inoculum concentration used in the second series. Pod-wall discoloration was observed, as before. It was again noted that the 4 nonpurpling isolates produced brown spots on some of the seeds, but no pod-wall discoloration. Seeds and pod walls from both check treatments were again unblemished.

Discolored seeds from both inoculation series were next plated out to determine if the fungi were present. Six discolored seeds from each isolate in both inoculation series were surface-sterilized and plated out on PDA supplemented with 50 ppm of the sodium salt of 2,4-dichlorophenoxyacetic acid.[8] Sterilization consisted of 30 seconds in 50% ethanol, followed by 1

TABLE 1.—*Purple discoloration of soybean seeds from pod inoculation with Cercospora isolates from several hosts*

Cercospora species	Source of isolates	Proportion of seeds discolored* Series I	Series II
	Isolates from leguminous hosts		
C. kikuchii	*Glycine max* (seed)	14/16	16/23
	Glycine max (seed)	17/18	13/19
	Glycine max (stem)	17/17	2/27
C. sojina Hara	*Glycine max* (leaf)	0/16	0/52
C. medicaginis Ell. & Ev.	*Medicago sativa* (leaf)	16/16	26/31
C. zebrina Pass.	*Trifolium incarnatum* (leaf)		30/43
	Trifolium pratense (leaf)	19/21	20/25
C. canescens Ell. & G. Martin	*Vigna sinensis* (stem)	21/22	15/24
C. cruenta Sacc.	*Vigna sinensis* (leaf)	0/14	0/52
	Isolates from nonleguminous hosts		
C. brachiata Ell. & Ev.	*Amaranthus retroflexus* (leaf)	22/27	29/30
C. capsici Heald & Wolf	*Capsicum frutescens* (leaf)	18/22	5/35
C. gossypina Cke.	*Gossypium hirsutum* (leaf)	19/20	21/25
C. malayensis F. L. Stev. & Solheim	*Hibiscus esculentus* (leaf)	19/21	9/27
C. ricinella Sacc. & Berl.	*Ricinus communis* (leaf)	21/22	1:/18
C. sesami Zimm.	*Sesamum indicum* (leaf)	18/21	17/20
	Sesamum indicum (stem)	14/14	24/24
C. sorghi Ell. & Ev.	*Sorghum halepense* (leaf)	0/8	0/36
	Sorghum vulgare (leaf)	0/12	0/52
Distilled water inoculation		0/17	0/42
No treatment		0/19	0/77

*Numerator, number of seeds discolored purple; denominator, number of seeds in inoculated pods.

minute in 1/1000 mecuric chloride. This was followed by 3 rinses through sterile distilled water. *Cercospora* was obtained from nearly every seed of all isolates in both inoculation series, except that the water-inoculated and no-treatment checks were sterile. The *Cercospora* colonies obtained from the *C. sojina*, *C. cruenta*, and *C. sorghi* seed appeared to be those respective species, while the colonies obtained from the purple-stained seed were indistinguishable from one another. These results show that seed infection took place, and that the discoloration was due to the presence of the fungi within the seeds.

During the experiments, marked similarities were noted between the *C. sojina* and *C. cruenta* isolates. The spore morphology and culture characteristics of the 2 isolates were quite similar. This similarity, plus the pod-inoculation reaction, indicated the possibility of a single species. A cross-inoculation experiment was therefore made.

Two varieties of cowpeas (White Acre and Monarch Blackeye) and 2 varieties of soybeans (Tanner and Lee) were used as hosts. Tanner is susceptible to *C. sojina*, and Lee resistant, whereas both White Acre and Monarch Blackeye are susceptible to *C. cruenta*. Plants were grown, 3 per 5-in. pot, and inoculated when 5 weeks old. Nine plants of each variety were sprayed with inoculum prepared from cultures of each of the 2 isolates. The inoculated plants were placed in a moist chamber for 48 hours, and transferred to a greenhouse bench.

Eleven days after inoculation, the Tanner soybean plants inoculated with *C. sojina* showed numerous and distinct frogeye lesion, whereas none of the other plants expressed symptoms. Eighteen days after inoculation, both varieties of cowpeas inoculated with *C. cruenta* showed numerous and distinct leaf-spot lesions, whereas those inoculated with *C. sojina* did not. Continued observation over several weeks did not detect any change in this pattern of infection. In spite of their similarities, it was evident that the 2 fungi were separate species.

Discussion.—The foregoing results show that purple stain of soybean seeds can be produced by several *Cercospora* species when they are inoculated into attached developing pods. The unwounded seed coats are penetrated and subsequent fungal growth produces purple-staining of the seeds.

Throughout the experiment it was observed that all the isolates that stained seeds purple also produced purple pigment in culture. None of the isolates that failed to stain seeds purple produced purple pigment in culture. This observation is similar to that made by Kilpatrick and Johnson[3] in studying seeds inoculated in culture. They reported that the isolates of *Cercospora* that stained seeds purple also produced purple pigment in PDA in the absence of seeds. This point is particularly pertinent in the case of *C. cruenta*, which in their tests produced purple stain and purple pigment, while it did neither in the present experiment. A possible explanation is that we were working with different strains. In all other cases where identical species were tested under both methods, results were similar. There is an obvious correlation between the production of purple pigment and the ability to incite purple stain of soybean seeds.

Besides producing purple pigment in culture, the purple-seed-staining isolates of *Cercospora* were indistinguishable in culture characteristics and had similar spore morphology. All these isolates possessed hyaline acicular conidia such as described for the type species, *C. apii* Fres. The nonstaining isolates, on the other hand, could be readily differentiated from the staining isolates on these points.

The fact that several morphologically similar *Cercospora* species can produce purple-staining of soybean seeds when inoculated into the host suggests the possibility of a single variable species. Lending credence to this possibility is the work of Johnson and Valleau[4] on *Cercospora* synonymy. They concluded from cross-inoculation study that species of *Cercospora* having the general morphological characteristics of *C. apii* were in fact a single species. In the present case, however, additional evidence, as indicated by the results of the cross-inoculation test with *C. cruenta* and *C. sojina*, will be necessary to prove the existence of a single variable species inciting the purple stain of soybeans.

It can be concluded that purple stain of soybean seeds can be incited by several species of *Cercospora* upon entrance into soybean pods. Whether these same *Cercospora* species incite purple stain of soybean seeds in the field is not known.

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INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF WHEAT BUNT IN THE PACIFIC NORTHWEST

II. EFFECT OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION BY SEED-BORNE SPORES[1]

E. L. Kendrick and Laurence H. Purdy[2]

SUMMARY

Seed of Red Bobs wheat, a susceptible spring wheat, was inoculated with spores of *Tilletia caries* (race T-5) and incubated at 5, 10, 15, 20, and 25°C in a Palouse Silt Loam soil maintained at 6 different moisture levels (9, 10, 11, 13, 18, and 24%). As the seedlings emerged from the soil at the various temperatures, they were removed, treated to prevent any further infection, and transplanted into greenhouse benches, where they were grown to maturity. The combination of 10°C and a 13% moisture level was the most favorable for bunt development. Bunt development was relatively high at the 11, 18 and 24% moisture levels, dropped off rapidly at 10% and was absent at 9%. Infection at all moisture levels was greatest at 10°C, and next greatest at 15° and 5°. Less than 5% bunt developed at 20°, and none developed at 25°.

A close relationship has long been recognized between certain environmental factors and infection of wheat plants by the bunt fungi (*Tilletia caries* (D.C.) Tul. and *T. foetida* (Wallr.) Liro). Among the more important factors are the moisture content and temperature of the soil, both of which influence spore germination and infection. Published reports fail to agree, however, on the optimums for greatest infection. The evident need for an evaluation of soil moisture and temperature in relation to other considerations (methods, soil-moisture terminology, host variety, pathogenic races) was brought out in a previous report,[3] dealing with the effect of soil moisture and temperature on spore germination by race T-5. This report is on the effect of soil moisture and soil temperature on infection by race T-5.

MATERIALS AND METHODS.—Seeds of the susceptible spring wheat variety Red Bobs were inoculated with spores of race T-5 of *T. caries* at the rate of 1 g of spores per 100 ml of seed. A Palouse Silt Loam soil was used that had a 1/3-atmosphere value of 24.0% and a 15-atmosphere value of 10.8%, both on a dry-weight basis.[3]. These soil-moisture constants were determined with methods described by Richards and Weaver.[4] The 1/3- and 15-atmosphere values correspond approximately to the field capacity and permanent wilting point (PWP), respectively, of the soil. The range between these two points represents the moisture available for plant growth. The 5 moisture levels (9, 11, 13, 18, and 24%) adopted in previous experiments[3] were used in these experiments, plus a 10% level.

To obtain the various moisture levels, a large sample of soil was screened and then air-dried, after which its moisture content was determined. Samples of soil were brought to a desired uniform moisture content by atomizing the required amount of water onto the soil sample in a rotating drum.[3] Moisture content in these samples varied from the calculated value by no more than 0.4%. Two-hundred-gram samples of the moistened soil were immediately placed in 1-pt. friction-top cans. Twnety-five inoculated kernels of wheat were placed on the surface of this soil mass and covered with 1 in. of the moistened soil. The friction tops were then placed on the cans to prevent loss of moisture by evaporation, and 2 seeded cans of each moisture level were incubated at 5, 10, 15, 20, and 25°C. The experiment was repeated 4 times.

When the seedlings in each can emerged through the soil surface, they were removed and washed free of soil by running water. Investigations, now in progress, concerning the time and establishment of infection by the bunt fungi have indicated that, at 10-25°, maximum infection occurred before emergence of the seedlings. However, by the end of 3 weeks little or no emergence of seedlings occurred at 5°C in combination with all moisture levels or at the 9% moisture level in combination with all temperatures. Seeds under these conditions were only slightly swollen, or had developed a short coleoptile 1/2–1 mm long. Studies of time and establishment of infection also indicated that no infection took place after 3 weeks at 5°C. Consequently, seedlings and ungerminated seed were removed from all cans at 5°C or 9% moisture after 3 weeks' incubation. On removal from the cans, all seedlings were dipped in a solution of Panogen 15 (2.2% cyano(methylmercuri)guanidine), 1:1000, for 2 minutes in order to prevent any further infection from spores still adhering to the seedlings. The seedlings were then planted in greenhouse benches and grown to maturity, at which time bunt percentages

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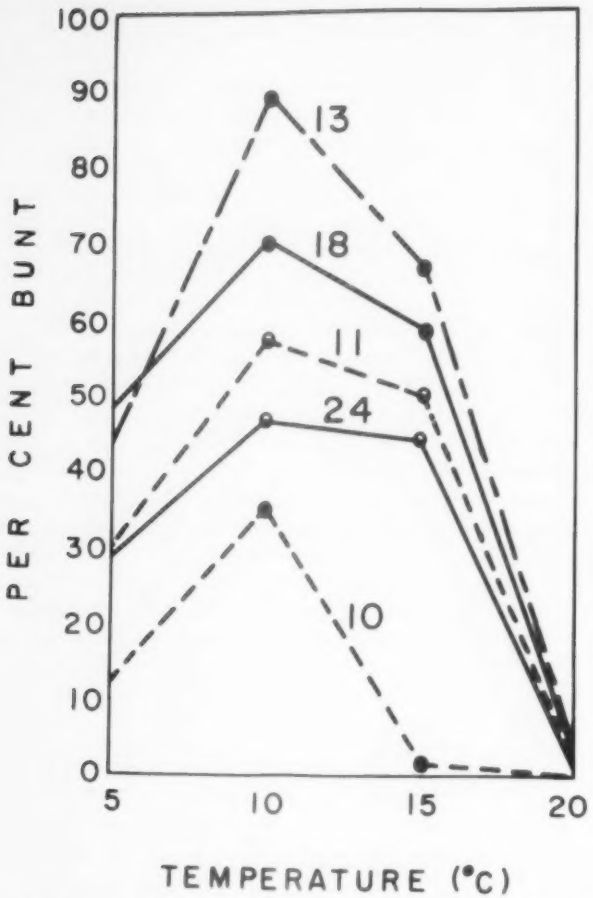

Fig. 1. Infection of Red Bobs wheat by seed-borne spores of *Tilletia caries* (race T-5) at 5 different soil moisture levels (10, 11, 13, 18, and 24%) and 4 different temperatures (5, 10, 15, and 20°C).

were determined .

Results.—The results, generally in agreement from the 4 experiments, are summarized graphically in Fig. 1. A moisture level of 13% and a temperature of 10°C were most favorable for bunt development.

Relatively high percentages of bunt developed at the 11, 18, and 24% moisture levels, which were, respectively, just above PWP, the midpoint of the available moisture range, and approximately field capacity. Infection dropped off rapidly at the 10% moisture level, and no bunt developed at the 9% moisture level at any temperature.

Infection was generally good at 5, 10, and 15° in the moisture range 11-24%. However, the influence of temperature was quite pronounced at the 10% moisture level. Infection level increased from 12% at 5° to 35% at 10°, and dropped sharply to a low of 2% at 15°. Less than 5% bunt developed at 20° at all moisture levels, and none developed at 25°. The results from the 9% moisture and from 25°C are not included in the graph.

Discussion.—These results, showing a pronounced effect of soil moisture and temperature on infection by seed-borne inoculum of race T-5, closely parallel results reported for the influence of these same factors on spore germination of this race. Generally, the soil moistures and temperatures that were optimum for infection were also optimum for spore germination.[3] Spore germination was equally good after 10 days at 5, 10, and 15° at soil moistures of 13-24%, but declined markedly at higher temperatures.[3]

Though spore germination was 50% at 20 and 25° at the 18 and 24% moisture levels,[3] infection dropped off rapidly at 20°, and none occurred at 25° at these same moisture levels. Thus, little or no infection developed even though spores germinated at these relatively high temperature and moisture levels. At 5°, spores germinated well after 10 days at all soil moisture levels of 11–24%.[3] However, infection was much lower at 5° than at 10° and at 15°, except at the 10% moisture level.

It seems evident that the spores of race T-5 can germinate over a relatively wide range of soil temperature and moisture, but that infection is affected more critically by temperature. It was shown that infection can take place in a Palouse Silt Loam soil with moisture contents ranging between field capacity and the permanent wilting point in a temperature range of 5–15°. However, a temperature-moisture combination of 10° and 13% was definitely the most favorable for infection. Further studies will take into account the interaction of these factors as they might influence infection of different varieties by different races of the bunt species.

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DIFFERENTIAL HOST AND SEROLOGICAL RELATIONSHIPS OF POTATO VIRUS M, POTATO VIRUS S, AND CARNATION LATENT VIRUS[1]

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SUMMARY

Potato viruses S and M showed significant differences in host range and symptomatology, and carnation latent virus would not infect solanaceous hosts. Leafrolling-mosaic-diseased Green Mountain, interveinal-mosaic-diseased Irish Cobbler, paracrinkle-carrying King Edward, and outwardly healthy Fortuna and D1102 potatoes were shown to be infected with virus M, and also with the more widespread virus S. Virus M from these sources was separated from virus S by passage through tomato or Saco potato. The different isolates of virus M differed in their abilities to incite complex diseases in several potato varieties, and they showed some variations in differential host symptomatology. Serologically, the virus M isolates were closely related, and were more distantly so to virus S and CLV. Cross-absorption tests indicated that virus S, virus M, and CLV each have a major antigenic fraction distinct from the others, while viruses S and M each have a distinct fraction in common with CLV. There also appears to be a small antigenic fraction common to all three, but the existence of any distinct fraction common only to viruses S and M is uncertain. The three probably had a common ancestor, but the differences seem to warrant classification as separate viruses rather than as strains.

Since the discovery of potato virus S, in Holland (5, 13), this and related viruses—in potatoes, and in one instance in carnation—have been reported from widely scattered parts of the world (1, 2, 8, 11, 12, 14, 15, 21, 22). Most of the reports appeared to deal with viruses identical to the Dutch virus S, but a number of exceptions caused some confusion. It has become increasingly apparent that these exceptions involve one or the other of 2 viruses that are slightly related to virus S: the carnation latent virus (CLV) (8) and potato virus M (2). CLV does not infect potatoes or other solanaceous hosts, and is thus readily distinguished from virus S. Virus M, however, is usually accompanied in potatoes by the more widespread virus S, and exists in a number of types that have only recently been shown to compose a closely related group, distinct from virus S. Since the 3 viruses have not been compared previously under one set of conditions, we have studied them with respect to host range and reactions, and serological relationships. Comparisons of their particle sizes, which are very similar (4), and of differences in transmissibility by insects (24) are reported elsewhere.

MATERIALS AND METHODS.—Virus S was obtained from a Wisconsin stock of U. S. D. A. Seedling 41956, and from a Fredericton stock of Arran Victory potato, except that one antiserum was made with virus S from Arran Victory potato at Braunschweig. Virus M was obtained from a number of different sources. Differences between some of these were on the order usually ascribed to virus strains, but we refer to them as "types" or "isolates" of virus M in order to avoid a multiplicity of strain names at this juncture. The leafrolling mosaic (LRM) (18) and interveinal mosaic (IVM) (17) types of virus M were from Green Mountain and Irish Cobbler potatoes, respectively, obtained from original stocks of E. S. Schultz (2, 20), and were cultured in plants of Seedling 41956; the paracrinkle type came from 2 lots of King Edward potatoes, respectively obtained from D. J. MacLeod, of Fredericton (2), and G. Cockerham, of Pentlandfield, Scotland (22); the D1102 type was obtained in a potato seedling from E. Köhler (11, 22); the Fortuna type in potatoes of this variety from Braunschweig (22); and the Bintje type in a leaf of *Datura Metel* L. received from A. Rozendaal and D. H. M. van Slogteren (15). CLV was supplied by B. Kassanis (8), and was maintained in plants of *Dianthus barbatus* L. (sweet william). The original sources of virus S and CLV were free of other known viruses, but all of the above sources of virus M, save the Bintje type, were contaminated with virus S. Isolates of virus M were obtained in the early part of our work by inoculation of tomato, a host immune to virus S (2, 14, 22), and were later cultured in Saco potato, which is considered immune to viruses S and X (1, 2).

Plant culture and inoculation techniques were similar to those described by Bagnall, Larson, and Walker (2), and seeds of the differential host plants were from the sources they used or from stocks newly obtained by R. H. Larson.

At Madison, the greenhouse temperature was held at 20°C. Day length was 16 hours during February and March, when most of the differential host work was done. There were some differences between symptom expression obtained at Madison and that obtained in supplementary tests at Fredericton or Braun-

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schweig, probably because of environmental differences.

Most of the serological antisera were prepared by injecting rabbits intravenously with partially purified and concentrated virus suspension, prepared as follows: Infective plant sap, first centrifuged 10 minutes at 1000 G, was shaken with half of its volume of peroxide-free ether, clarified by centrifuging, then shaken with a similar volume of carbon tetrachloride and reclarified. The supernatant, largely freed of its chlorophyll and fat components, was centrifuged 4 hours at 20,000 G, and the soluble fraction of the pellet was taken up in a volume of 0.15*M* phosphate buffer (pH 7) representing 1/20 of the original sap volume. This preparation invariably showed anisotropy of flow. After being dialyzed overnight against 0.15*M* sodium chloride (pH 7.5), it was used for 4 injections in the amounts of 0.5, 1, 1.5, and 2 ml at 3-day intervals and after waiting 3 weeks, 2 further 2 ml injections 3 days apart, then a 10-day interval was allowed before bleeding. Some of the earlier antisera were prepared by injecting the rabbits with infective plant sap that had been clarified, only, by centrifuging for 30 minutes at 6000 G. Although this simple method produced some good antisera (IVM_1 and CLV_1, below), the use of partially purified and concentrated virus suspension was more reliable. The purification procedures also eliminated sap components that appeared to cause local reactions and systemic shock in the rabbits.

Serological precipitin tests were set up as follows: Potato sap containing virus S, tomato or Saco potato sap with virus M, and sweet william sap with CLV were heated 10 minutes at 45°C, cooled, and centrifuged 20 minutes at 1000 G, to clarify. To delay nonspecific precipitation, the clarified saps were diluted to 1/10 strength with 0.85% saline. Dilute virus preparation in the amount of 0.5 ml was placed in each serological tube, and 0.5 ml of appropriately diluted antiserum was added. The tubes were agitated and placed ¼ in. deep in a water bath held at 40°C. Precipitation was observed for 2–4 hours, the tubes placed overnight in a refrigerator at 4°C, and returned to the bath for an hour of periodic observation the following day. Control tubes containing virus and saline as well as antiserum and healthy plant sap, were also observed. Where nonspecific precipitation interfered with the results of a given test, the entire test was rejected. Readings in these tests were concerned with titer of the antiserum against homologous and heterologous viruses, and are expressed as the inverse of the smallest fractional concentration of liquid antiserum that resulted in visible precipitation.

RESULTS.—*Reactions of differential hosts.*—Plants of a number of differential hosts were inoculated with virus S in sap from Seedling 41956; the LRM, IVM, paracrinkle (both sources), Fortuna, and D1102 isolates of virus M from tomato or Saco potato; and CLV from sweet william. The symptoms on these plants were compared with those on control plants, and attempts were made, whether or not any abnormal symptoms appeared, to recover or detect the virus, and so learn if infection had occurred. For this purpose, *Nicotiana debneyi* Domin. was used as an indicator plant for virus S, *Datura metel* for virus M, and the serological precipitin test was used to detect CLV. CLV incited no symptoms on any of the differential host plants used, and could be shown to infect only sweet william. Sweet william plants, on the other hand, appeared to be immune from all isolates of virus S and virus M. Several plants commonly used as differential hosts for potato viruses, including *Capsicum annuum* L., *Nicotiana glutinosa* L., *N. tabacum* L. var. Havana 38, were immune to both virus S and virus M. Tomato, though showing no symptoms, was susceptible to all isolates of virus M, but was immune to virus S. Symptoms incited by virus S and the different isolates of virus M on the other host plants are summarized below:

1) *Chenopodium album* L.—Virus S incited small yellow local spots, which were later surrounded by green bands on mature yellow leaves (2, Fig. 10). None of the virus M isolates incited symptoms on this host.

2) *Datura metel.*—Virus S became systemic, usually without symptoms, but occasionally incited a faint, transient mottling. All isolates of virus M, on the other hand, incited a local and systemic chlorotic or necrotic spotting, frequently accompanied by distortion of the upper leaves (Fig. 1). The systemic spotting was less pronounced on plants infected with the D1102 and Fortuna isolates of the virus. When the plants were infected with viruses S and M simultaneously, symptoms resembled those due to virus M, but were sometimes more severely necrotic, and during the earlier stages of infection, only virus M could be recovered from upper leaves.

3) *Gomphrena globosa* L.—Virus S incited no symp-

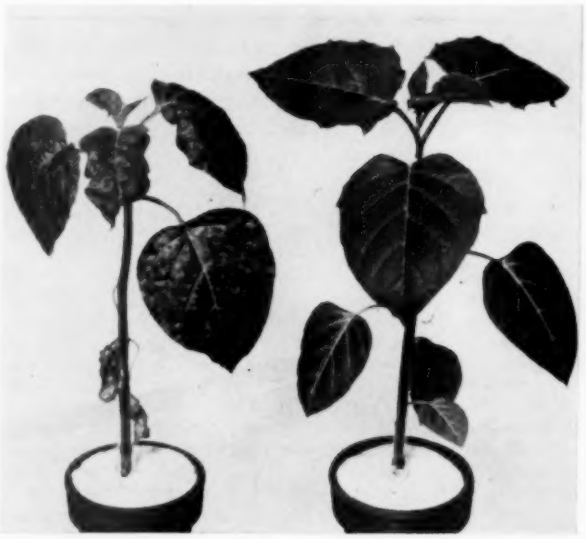

Fig. 1. *Datura metel* infected with the interveinal mosaic isolate of virus M (left) and uninoculated control (right).

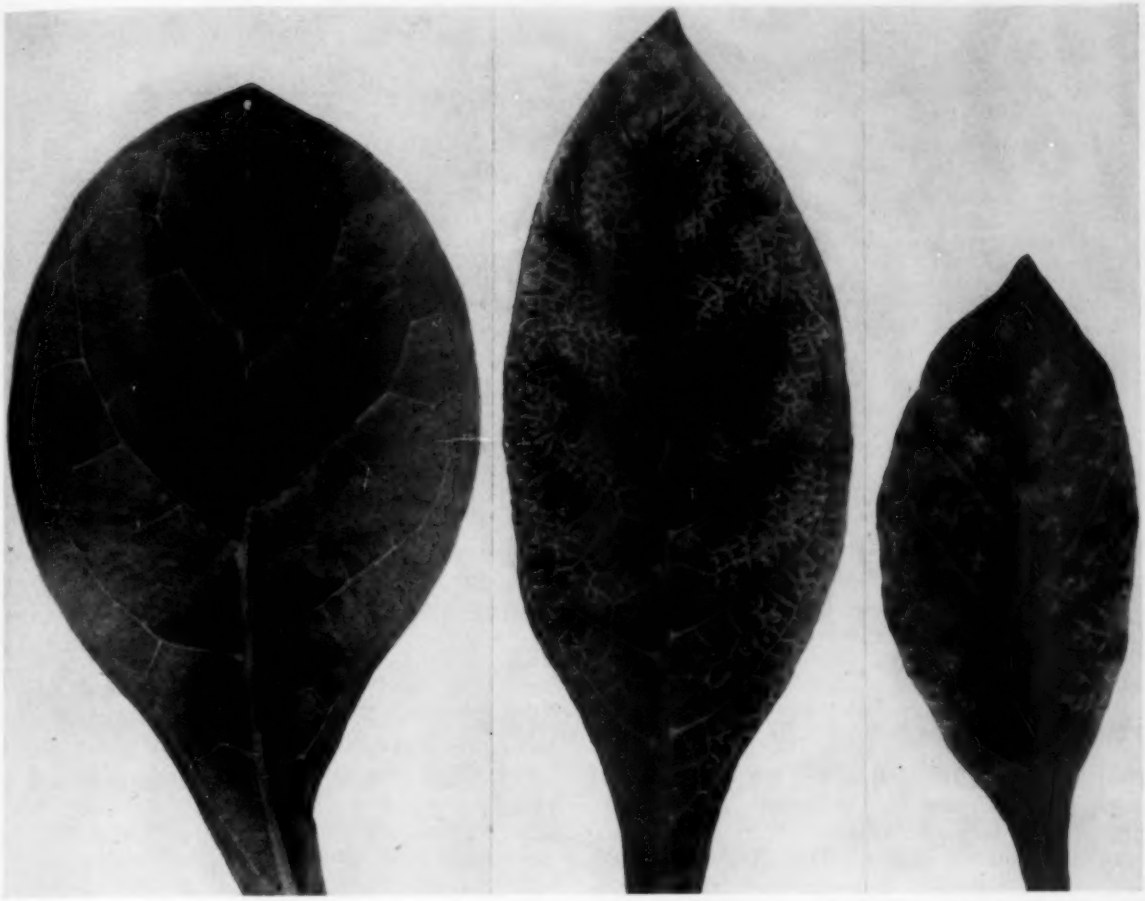

Fig. 2. Leaves from a single plant of *Nicotiana debneyi* infected with virus S. Inoculated basal leaf (left) is symptomless while the middle and upper leaves show typical vein clearing.

toms, nor did the LRM, IVM, or paracrinkle isolates of virus M. All of these viruses, however, infected this host locally, and could be recovered from inoculated leaves. The D1102 and Fortuna isolates of virus M incited small chlorotic spots, which appeared locally about 10 days after inoculation and were later surrounded by reddish borders (2, Fig. 17).

4) *Nicotiana debneyi*.—Virus S incited no local symptoms, but, about 20 days after inoculation, a veinclearing was evident on the first leaf above the basal whorl, spreading from the tip toward the base of the leaf (Fig. 2). Successively higher leaves developed a similar veinclearing, which was eventually replaced by an interveinal or a veinbanding mottle, and finally by a yellowing of the leaf lamina, often accompanied by necrotic lesions in the interveinal areas. All isolates of virus M incited irregular brown, ring-like local lesions varying in severity with the different isolates (Fig. 3), but no systemic symptoms appeared. Only rarely could virus M be recovered systemically from this host, and it was an excellent differential for the selection of virus S from mixed inoculum.

5) *Solanum rostratum* Dunal.—Virus S incited a necrotic spotting of inoculated leaves within 20 days. These necrotic lesions continued to appear systemically on successively higher leaves (2, Fig. 11). The plants were somewhat stunted, but the stems and petioles otherwise appeared normal, and apical development continued. All isolates of virus M incited a systemic streaking of the stem and petioles, beginning about 30 days after inoculation (2, Fig. 15).

6) *Vicna sinensis* Endl. (cowpea, var. Black).—Virus S incited no symptoms. All isolates of virus M incited reddish local lesions on the inoculated primary leaves, with occasional streaks of red along the veins (2, Fig. 13).

7) *Cyamopsis tetragonaloba* (L.) Taub. (guar).—Virus S incited small necrotic local lesions in 6–10 days. All isolates of virus M incited similar lesions, usually in 4–6 days (2, Fig. 9).

Virus M and virus S in potatoes.—The earliest report of a disease that we can associate with virus M or virus S was the leafrolling mosaic of potatoes described by Schultz and Folsom in 1923 (6, 18, 19). Other early reports include paracrinkle (16), inter-

Fig. 3. Basal leaves of *Nicotiana debneyi* inoculated with the interveinal mosaic (left), Fortuna (center), and paracrinkle (right) isolates of virus M. (Compare with virus S in Fig. 2.)

veinal mosaic (2, 18), and rolling mosaic (10). We cannot, however, refer to a leafrolling mosaic virus, a paracrinkle virus, or an interveinal mosaic virus, because the potatoes originally exhibiting each disease were infected with more than one virus. This is probably true also of the rolling mosaic disease which seems to have been associated with a virus similar to our D1102 and Fortuna isolates of virus M. In addition to virus M, virus S was recovered from our LRM Green Mountain, IVM Irish Cobbler, and paracrinkle-carrying King Edward, as well as from our D1102 and Fortuna potatoes. The former two were infected with virus X also, and this had first to be eliminated from the complexes by passage through virus-X-immune potato seedlings. Virus S was separated from virus M in each case by systemic passage through *Nicotiana debneyi*. The isolates of virus S obtained in this way appeared quite similar to our virus S from Seedling 41956 with respect to symptoms produced on *N. debneyi*, *Solanum rostratum*, and guar. Virus S is essentially latent in plants of Seedling 41956; and most of the other potato varieties we have tested, including "healthy" Arran Victory, Green Mountain, and Irish Cobbler, also carry a similar type of this virus without prominent symptoms. The variety Saco is apparently immune to virus S (1, 2). It was of interest, however, to determine the reactions of certain potato varieties to the different isolates of virus M, alone or in addition to whatever latent viruses might be present.

By top-grafting with infected tomato or Saco potato scions, each isolate of virus M was inoculated to plants of the varieties listed below. Symptoms were observed on the grafted plants, and where time permitted, on secondary plants grown from tubers of these. There were differences in varietal reactions, but we have not determined whether these are due to factors inherent in the varieties, or to influences of secondary viruses or their strains, present in several of the varieties.

1) *Saco.*—The LRM, Fortuna, and D1102 isolates of virus M incited a faint veinclearing on leaves of side shoots that developed on grafted Saco plants. Other isolates incited no symptoms. A faint interveinal mottle appeared on the leaves of secondary plants infected with the LRM isolate, and a veinclearing on leaves of those infected with the Fortuna and D1102 isolates. No symptoms were incited by the other isolates.

2) *Seedling 41956.*—All plants were infected already with virus S. The two paracrinkle isolates of virus M incited a wavy distortion and mottling on leaves of grafted plants, while the other isolates incited no symptoms. The distortion and mottling was also seen on secondary plants infected with the paracrinkle isolates, becoming more severe as the plants grew older. Leaves of secondary plants infected with the LRM isolate had a slightly harsh, cupped, and wavy appearance, while plants infected with the other isolates and healthy plants had leaves that were flat or curved slightly downward about the edges.

3) *Arran Victory.*—Plants of this variety, also infected with virus S, behaved very similarly to those of Seedling 41956. The leaf distortion, however, appeared on both the grafted and secondary plants infected with the LRM isolate.

TABLE 1.—*Titers of virus S, virus M, and CLV antisera tested against different isolates of these viruses*

Antiserum	Virus S		Virus M						CLV
	41956	A. Vict	Para	Fort	D1102	IVM	Bin	LRM	
S (41956)	8,192		16	16		16		8	128
S (A. Vict)	2,048	4,096	0[a]	16	0	16			16
M (Para)	128	64	4,096	2,048	1,024	1,024			64
M (Fort)		32	2,048	2,048	2,048	2,048			
M (D1102)	16	64	4,096	4,096	2,048	2,048			
M (IVM_1)	16	16	2,048	2,048	1,024	1,024			256
M (IVM_2)	64		8,192	8,192		8,192	8,192	8,192	256
M (Bin)	64	64	2,048	2,048	1,024	2,048			32
CLV_1	128	128	128	128	128	128			2,048
CLV_2	512	512	256	128		512	256	128	32,768

[a]Less than 8.

4) *Green Mountain.*—Stalk plants already carrying viruses S and X, infected with the LRM isolate, developed a severe curling distortion of their leaves, accompanied by a faint mottling, and plants infected with the IVM isolate developed a slight rolling of the upper leaves without mottling. Plants infected with the other isolates were relatively symptomless. No secondary plants were available, excepting older stocks infected with the LRM and IVM isolates. The former showed the curling and interveinal mottle as above, while the latter developed an interveinal mottle with a slight rolling of the upper leaves.

5) *Irish Cobbler.*—Grafted plants, also carrying viruses S and X, infected with the paracrinkle isolate of virus M, developed a distortion and mottle similar to that seen on Seedling 41956, and plants infected with the LRM, IVM, and Bintje isolates developed a faint interveinal mottle. The only secondary plants available were the original stocks infected with the IVM isolate. These plants showed interveinal mottling, accompanied on older leaves by some distortion that appeared to be due to uneven growth about small necrotic lesions.

Serological experiments.—1) *Relationships between the different virus isolates.*—In a preliminary study of serological relationships, available antisera were titrated against the different virus isolates. Two virus S antisera had been prepared with virus from Seedling 41956 and Arran Victory potatoes, respectively. Of six virus M antisera, the paracrinkle, D1102, and Fortuna types were prepared with concentrated virus from infected tomato; one IVM serum (IVM_1) had been prepared with clarified potato sap in 1956 (2), and preserved by freezing; another IVM serum (IVM_2) was prepared with concentrated virus from Saco potato; and one serum was received from A. Rozendaal and D. H. M. van Slogteren (15) in lyophylized condition, having been prepared with an isolate of virus M from Bintje potato. There were two CLV sera, the CLV_1 serum having been prepared with clarified sap from infected sweet william plants, and the CLV_2 with concentrated virus from the same source. Thus, there were 10 antisera prepared with 8 different virus isolates. These antisera were tested against the different isolates of virus S, virus M, and CLV, as shown in Table 1, in which the titers are compared. Some tests were omitted because a particular antiserum or virus was unavailable at the time.

The titers of the different antisera against homologous virus isolates were, for the most part, within the range of 1024-8192, but the homologous titer of the CLV_2 serum was 32,768. Homologous titer, probably the best measure of antiserum strength, must be taken into account when comparisons involve 2 or more antisera, for homologous and heterologous titers appear to be related, if not directly proportional. It is also essential to consider the reciprocal titers in comparing any given pair of virus isolates and their respective antisera. In most cases, for example, virus M antisera had higher titers against virus S isolates than did the particular virus S antiserum against the virus M isolate in the reciprocal test.

When the antisera strengths and reciprocal titers are taken into account in evaluating the data in Table 1, we find that virus S, virus M, and CLV are 3 slightly related, but nonetheless clearly distinguishable viruses. An isolate of one virus will be seen to possess a close antigenic relationship to other isolates of that virus, and a comparatively slight relationship to an isolate of either of the other two viruses. There is no evidence that any of the individual isolates are intermediate between two of the viruses. There is some evidence, however that, serologically, virus S and virus M are related slightly more closely to CLV than to each other. This is shown by the fact that, when CLV or its antiserum is one of the reactants, serum titers are greater than the titers of S-serum against virus M or M-serum against virus S.

2) *Cross-absorption tests.*—Our study of the serological relationships was carried a step further in cross-absorption tests. S(41956), M(IVM_2), and CLV_2 antisera were each absorbed separately with concentrated virus S(41956), virus M(IVM), and CLV, making 9 absorption tests. Each of the 9 absorbed antisera, plus 3 control antisera, were then titrated against each of the 3 viruses, making a total of 36 titer determinations.

In the absorption it was necessary to ensure that specific antibodies were completely removed. Each of the 9 cross-absorption tests was therefore repeated 4 times with the proportion of antiserum to concentrated virus suspension adjusted to 1:1, 1:4, 1:16, and

TABLE 2.—*Titers of virus S, virus M, and CLV antisera, unabsorbed and absorbed with each of the three viruses*

Antiserum	Absorbing virus	Test virus: Virus S (41956)	Test virus: Virus M (IVM)	Test virus: CLV
S (41956)		8,192	16	128
	S	0	0	0
	M	8,192	0	64
	CL	8,192	0	0
M (IVM_2)		64	8,192	256
	M	0	0	0
	S	0	8,192	128
	CL	0	4,096	0
CLV_2		256	128	8,192
	CL	0	0	0
	S	0	128	8,192
	M	128	0	8,192

1:64. Preliminary tests had shown that the minimum amount of our virus suspensions that would give complete absorption was within this range. The above ratios were prepared by mixing fixed amounts of concentrated virus suspension (usually 1 ml) with equal amounts of appropriately diluted antiserum. The mixtures were incubated 4 hours at 40°C and kept overnight at 4°C. Excess virus, as well as the precipitate, was removed by heating 10 minutes at 65°C and centrifuging 30 minutes at 1000 G. Control antisera were passed through the incubation, heating, and centrifuging procedures, but were not absorbed, for their titers against healthy plant sap were extremely low.

It was noted that, as the relative amount of absorbing virus was increased, homologous absorption gradually reduced, and finally eliminated, the titers against homologous virus. Where the titer of unabsorbed CLV_2 serum against CLV was 32,768, the titer of 1:1 absorbed serum was 8192; that of 1:4 absorbed serum, 128; and the titers of 1:16 and 1:64 absorbed serum below the level that could be measured with such dilute sera. The respective homologous titers of virus M antiserum were 8192 for the control, 256 for serum absorbed 1:1 with virus M, 64 after 1:4 absorption, and undetectable after absorptions at 1:16 and 1:64. With virus S antiserum, the homologous titer was 8192 for the control, 512 after 1:1 absorption with virus S, 128 after 1:4 absorption, and undetectable after 1:16 and 1:64 absorption. Quite similarly to the above, heterologous absorption reduced and eliminated the small titers against the absorbing virus itself, though it had little effect on the large homologous titers, even at the 1:64 serum-to-virus ratio. Heterologous absorption usually reduced titers against the third virus to a certain level, ordinarily at the 1:4 serum-to-virus ratio, but not further with excess virus.

When the titer in a given test was reduced by each successive increase in the proportion of absorbing virus and finally became undetectable, the titer of completely absorbed serum was assumed to be zero. When the titer was not reduced at all, or leveled off at a detectable value, this value was taken to represent the titer of completely absorbed serum. Owing to dilution factors, the lowest titer that we found practical to read was 16. The titer of unabsorbed CLV_2 antiserum against the homologous virus was reduced in tabulation from 32,768 to 8192, in order to facilitate comparisons (Table 2). Other CLV_2 serum data were reduced proportionately.

As compared to titers of 8192 against homologous virus, the titers of the 3 unabsorbed antisera against heterologous virus ranged from 16 to 256. Those involving CLV or its antiserum, as in the previous experiment, were on the higher side of this range, whereas the virus S serum had its lowest titer, 16, against virus M, and virus M serum had its lowest titer, 64, against virus S.

In every case, as described above, we were able to eliminate the large titer against homologous virus by absorbing with this virus itself, indicating that absorption was complete. In only one case was the large titer against the homologous virus reduced by heterologous absorption, a one-step reduction in titer of virus M serum absorbed with CLV. In each of 6 tests, heterologous absorption eliminated the small titer against the absorbing virus itself. There were 6 cases also, in which serum was absorbed with heterologous virus and tested against the third virus. When virus M serum was absorbed with virus S, virus S serum with virus M, and CLV serum with virus M, a one-step reduction in titer against the third virus was effected. Absorption of CLV serum with virus S left the titer against virus M unchanged. Absorption of virus S serum with CLV eliminated the titer against virus M, and absorption of virus M serum with CLV eliminated the titer against virus S.

The over-all results of the cross-absorption tests indicate that each of the 3 viruses has a major antigenic fraction distinct from the others, and that viruses S and M each has a distinct fraction in common with CLV. There also appears to be a small fraction common to all three, but the existence of any distinct fraction common only to viruses M and S is uncertain.

DISCUSSION.—Our differential host and serological tests show clearly that virus S, virus M, and CLV are each a distinct entity, and that each can be isolated from a mixture without resorting to chance-like procedures such as dilution or local-lesion recoveries. Of the three, only virus S ordinarily becomes systemic in *Nicotiana debneyi*, only virus M infects tomato or Saco potato, and only CLV infects sweet william.

The different isolates of virus M incite slightly different symptoms in several of our test plants, as well as obviously different symptoms in *G. globosa* and several potato varieties. We have, however, avoided any attempt to set up a system of "strains" of this virus until these symptoms and serological specificity can be examined more critically. Also, a number of new isolates of virus M that have come to our attention will need to be considered.

Much of the confusion regarding these viruses, mentioned in our introduction, derives from the fact that virus M in potatoes is ordinarily accompanied by virus

S. Virus S is so widespread that, in the absence of cross-protection between the two, this would seem inevitable.

Serological comparison between such a mixture of virus S and virus M in King Edward potato, and virus S alone in Arran Victory potato, led Kassanis (8) to the conclusion that the "paracrinkle virus" (actually the mixture) was much more closely related to virus S than the paracrinkle isolate of virus M really is.

By serological tests, Rozendaal and van Slogteren (15) detected viruses S and M in "crinkle-diseased" Fortuna potatoes, but only virus S in "healthy" clones. Inoculum from both "crinkle-diseased" and "healthy" Fortuna plants incited reddish local lesions on *G. globosa*, a host that did not react in this way to other isolates of virus S. They found it necessary to postulate that a fourth member of the M-S-CLV group—an "abberrant strain" of virus S that incited local spots on *G. globosa*—was present in both types of Fortuna, and that only the "crinkle-diseased" plants contained virus M. These workers kindly supplied us with "healthy" and "crinkle-diseased" leaves of Fortuna. We have isolated virus S from both Fortuna sources by systemic passage through *N. debneyi*, and virus M from the "crinkle-diseased" source, but not from the "healthy" source, by infection of tomato. Although the virus M incited local spots on *G. globosa*, neither isolate of virus S would do so. It is possible that there are conditions under which the virus S isolates from Fortuna would incite such spots, or that there is a further virus involved. Nevertheless, in this and other tests reported in our results, incitement of local lesions on *G. globosa* by Fortuna inoculum has always been associated with the virus M in this source.

In designating which viruses are isolates of virus M, we have considered the close serological relationships, similar host ranges, and predominantly similar host reactions amongst these isolates. Less weight has been accorded to peculiarities in symptomatology such as the incitement of local spots on *G. globosa* by the Fortuna and D1102 isolates, but not by the other isolates of virus M. Such differences do not justify a distinction between isolates on the same order as we distinguish between virus S and virus M. Similar reasoning would apply to variants of virus S or CLV, and we do not consider that any fourth member of the S-M-CLV group has been described. Apart from this one difficulty, Rozendaal and van Slogteren (15) found the distinction between virus S and virus M (2) to be quite practical, for they encountered virus M in potatoes that had been maintained free of virus S.

In its recent review of virus nomenclature, the Review of Applied Mycology (7) gives equal status to the "leafrolling mosaic virus" and the "paracrinkle virus," while relegating virus S to the status of a strain of the latter. Evidence now indicates that paracrinkle is for all practical purposes a synonym of leafrolling mosaic, and that both of these diseases are incited by complexes of virus S and virus M.

The three-sided relationship between potato virus S, potato virus M, and the carnation latent virus appears to be similar in some respects to the relationship between cucumber virus 3 (with which we include the closely related cucumber virus 4) and the tobacco mosaic virus. Bawden and Pirie (3) showed that these latter two viruses had some antigenic fractions in common, but that their relationships to each other differ more widely than the commonly reported virus "strain" relationships. They appeared to favor the idea that the two viruses had a common origin. Knight (9) disputed "strain" relationship between cucumber virus 3 and tobacco mosaic virus, but was noncommittal on the question of whether the two viruses had a common ancestor or arose independently.

Another virus relationship that is possibly similar to the S-M-CLV type is that between the German sweet clover and pea stunt viruses, described by Wetter and Quantz (22).

It seems probable that virus groups such as those under discussion are more common than had been supposed, but it is obvious that the nomenclature of plant viruses has developed without taking them into account. There may be a growing need for some system of classification which will show that viruses in groups of the S-M-CLV type are related, yet avoid having the significance of their relationship lost amongst a host of artificial groups or the identity of the individual viruses obscured by a multiplicity of "strain" names. Such a system would recognize groupings analogous to true genera and species. We have, however, only a few virus groups of this type, and the basic nature of their relationships is little understood. An intensified study of virus relationships appears, therefore, to have priority over proposals for new systems of classification and nomenclature.

In absence of a better system of classification, we suggest that virus S, virus M, and CLV should be regarded as distinct viruses and should retain separate names. Since they are serologically related and have similar particle size, it seems probable that they arose from a common ancestor and could be regarded as members of a single group.

As material for study of virus relationships and evolution, the S-M-CLV group has significant features. These include the serological relationships; the same particle size; the overlapping host range, though differing host reactions for virus S and virus M; the differences in insect transmission within the group (15, 17, 23); and differences between virus S and virus M in thermal inactivation (2, 22).

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SIZE AND SHAPE OF THE PARTICLES OF POTATO VIRUS S, POTATO VIRUS M, AND CARNATION LATENT VIRUS[1]

J. Brandes,[2] C. Wetter,[2] R. H. Bagnall,[3] and R. H. Larson[4]

SUMMARY

Electron micrograph measurements of particles of virus S from 4 different potatoes free from other known viruses, 4 isolates of virus M (i.e., leafrolling mosaic, interveinal mosaic, D1102, and paracrinkle), and the carnation latent virus from sweet william, have shown that the 3 viruses have very similar lengths with an estimated diameter of 12–13 mμ. The normal lengths of particles of virus S was 657 mμ (82%), virus M was 651 mμ (80%), and the CLV was 652 mμ (68%). Some double-length particles and others that were about half of the normal length were observed. It is proposed that the 3 be classified as separate viruses within a single group.

Wetter and Brandes (7) made electron micrographs of particles of potato virus S from 12 different sources, and reported the virus to be rod-shaped, with a normal length of 652 mμ and a diameter of 12–13 mμ. Their plant sources for the virus S included D1102, Fortuna, and King Edward potatoes, all of which were thought to contain only strain variants of virus S. It has since been shown that each of these three potatoes contains both the common virus S and potato virus M (1, 2), the latter apparently being responsible for the "variant" properties. Although virus S and virus M have a slight serological relationship, significant differences in serological, differential host, and physical properties distinguish them.

Kassanis (4) showed that potato virus S also has a slight serological relationship with the carnation latent virus (CLV), although the two have no known hosts in common.

Bagnall, Wetter, and Larson (2) recently reviewed the serological differential-host properties of virus S, virus M, and CLV, and suggested that the three should be regarded as separate viruses within the same group.

With the relationships of these three viruses better understood, it was of interest to make new particle measurements. This was done with several different isolates of virus S and virus M, as well as with one of CLV. The results, which indicate that normal particle lengths are nearly the same, are reported here.

MATERIALS AND METHODS.—Potato virus S was obtained from greenhouse-grown plants of 4 different potatoes, including Arran Victory, Erdgold, Industrie, and U.S.D.A. Seedling 41956. All plants were tested serologically for the presence of virus S and the absence of virus M, using Arran Victory S-antiserum and D1102 M-antiserum, described by Bagnall, Wetter, and Larson (2). In addition, it was ascertained by differential-host tests that these potatoes were free of potato viruses A, F, X, and Y.

Potato virus M was variously obtained from plants of *Datura metel* L., *Gomphrena globosa* L., *Solanum demissum* L., and tomato, infected with one or another of the leafrolling mosaic, interveinal mosaic, D1102, or paracrinkle isolates used by Bagnall, Wetter, and Larson (2). The carnation latent virus was also that used by those authors, originally obtained from B. Kassanis (4), and cultured in *Dianthus barbaratus* L. (sweet william).

Johnson's exudate method (3) was used for preparing electron microscope grids. These preparations were shadow-cast with platinium-iridium or palladium, and pictures of the particles were taken with an electrostatic electron microscope (EM 8/2 AEG/Zeiss). The original negative magnification, 5320, was enlarged to 40,000 for measuring (Fig. 1). The original magnification was established by measuring the known spacing of a silicon oxide replica of a diffraction grating (5). This should give an error of less than 5%.

RESULTS AND DISCUSSION.—Data from particle measurements of each virus isolate were assembled into classes with a range of 25 mμ (i.e., 637.5–662.5=650), covering an over-all range of 300–1500 mμ. Particles of all 3 viruses, from whatever source, tended to have well-defined distribution maxima within the 650 mμ (single-length) and 1300–1325 mμ (double-length) classes. Further, when cumulative percentage curves were drawn on logarithmic probability paper, Hazen straight lines resulted indicating Gauss-distribution (6) about the maxima. The mean particle length within each distribution maximum was calculated from data on classes containing particle numbers clearly above the general level. Particles in classes outside such maxima were assumed to be fragmentary. The mean particle length within the main distribution maximum has been defined as normal length (7). We do not necessarily imply that the virus particles vary in length, for we have not ruled out the possibility that the Gauss distribution has been brought on by technical errors. Nevertheless, the mean particle length within the distribution maximum probably gives as

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good a measure of particle length as our technique will provide.

The results are summarized in Table 1, which gives the number of particles observed, the percentage of

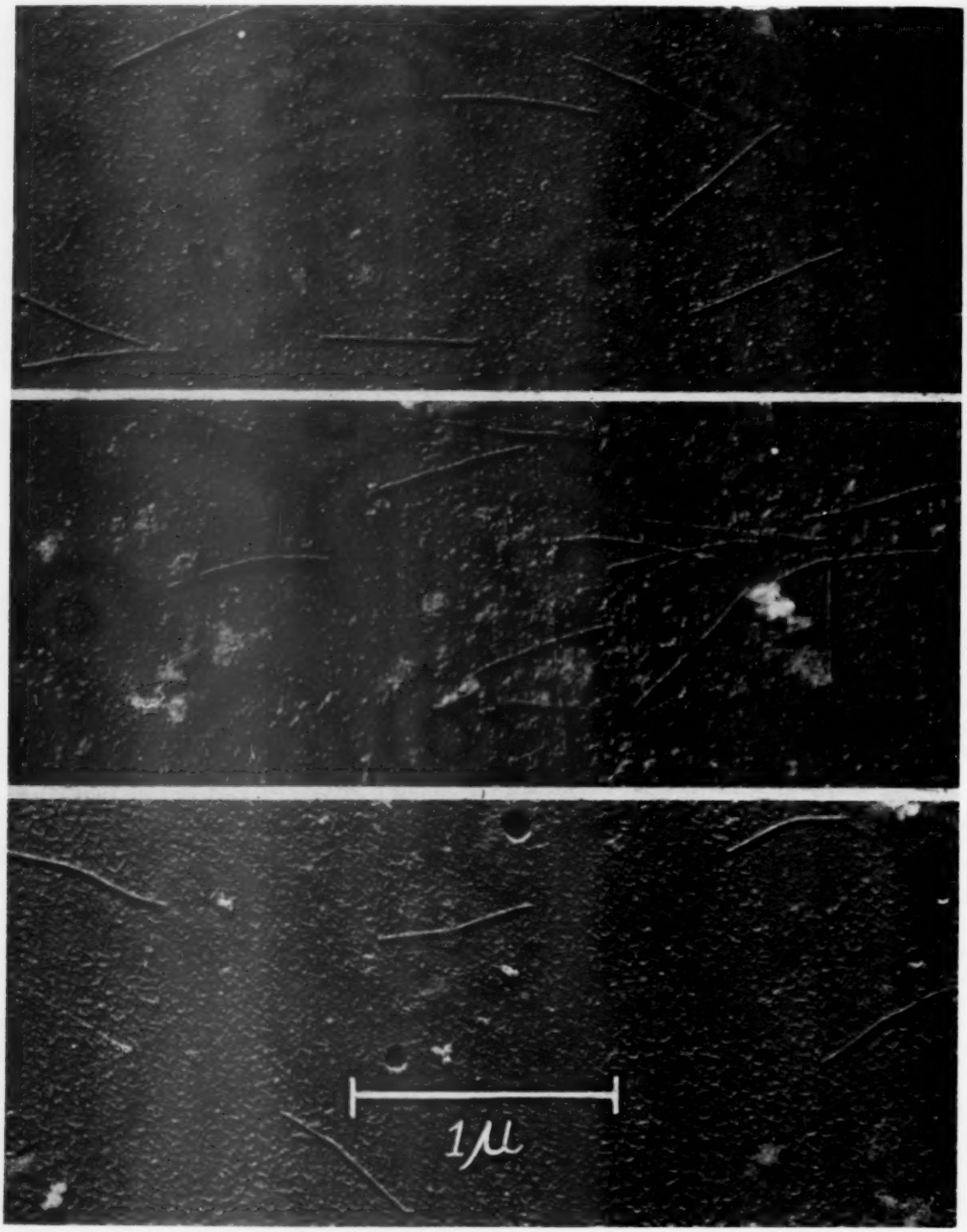

Fig. 1. Electron micrographs of potato virus S from potato Seedling 41956 (top); potato virus M (interveinal mosaic isolate) (middle); and carnation latent virus (bottom). (40,000×).

these falling within the normal-length curve, and the normal particle length for each virus isolate, as well as for virus S and virus M. In addition, the percentage within the double-length curve and the double-length mean is given for each of the three viruses. It should be noted that curves plotted from larger numbers of particles (i.e., virus S and virus M totals) tended to have broader bases than did curves plotted from smaller numbers, and the former consequently encompassed larger percentages of the observed particles. The actual distribution of particle lengths within classes for all particles of each of the three viruses is presented graphically in Fig. 2.

The normal lengths of the virus S particles ranged from 649 to 658 mμ for the different isolates, with a mean of 657 mμ for the virus S isolates combined. Eighty-two per cent of the particles measured fell within the curve about this mean. Five per cent of the particles fell within the curve about the double-length mean of 1310 mμ. Similarly, the normal particle lengths of virus M isolates ranged from 649 to 656 mμ, with an over-all mean of 651 mμ. Eighty per cent of the virus M particles fell within the curve about this mean, while 6 per cent were within the curve about the double length mean of 1299 mμ. Sixty-eight per cent of the CLV particles were within the curve about the normal length of 652 mμ, and 6 per cent were within the curve about the double length mean of 1328 mμ.

There was some indication of a distribution maximum with a mean of about 325 mμ, or one-half the normal length in the virus M and CLV data. The curve about this mean contained 4% of the virus M particles and 11% of the CLV particles. The significance of this "half-length" distribution maximum is uncertain.

It is apparent that potato virus S, potato virus M, and CLV have very similar particle lengths. Particles of the three viruses also had very similar widths in our electron micrographs. By comparison with the width of particles of tobacco mosaic virus, which are generally considered to approximate 15 mμ in diameter, we estimated the diameter of each of the above three viruses to be 12–13 mμ.

This almost exact similarity in size lends weight to the proposal to classify these three viruses within a single group (2), which previously had been based on only a small correspondence of antigenic fractions. We must then look beyond the gross morphology to explain the large antigenic differences as well as the differences in other properties.

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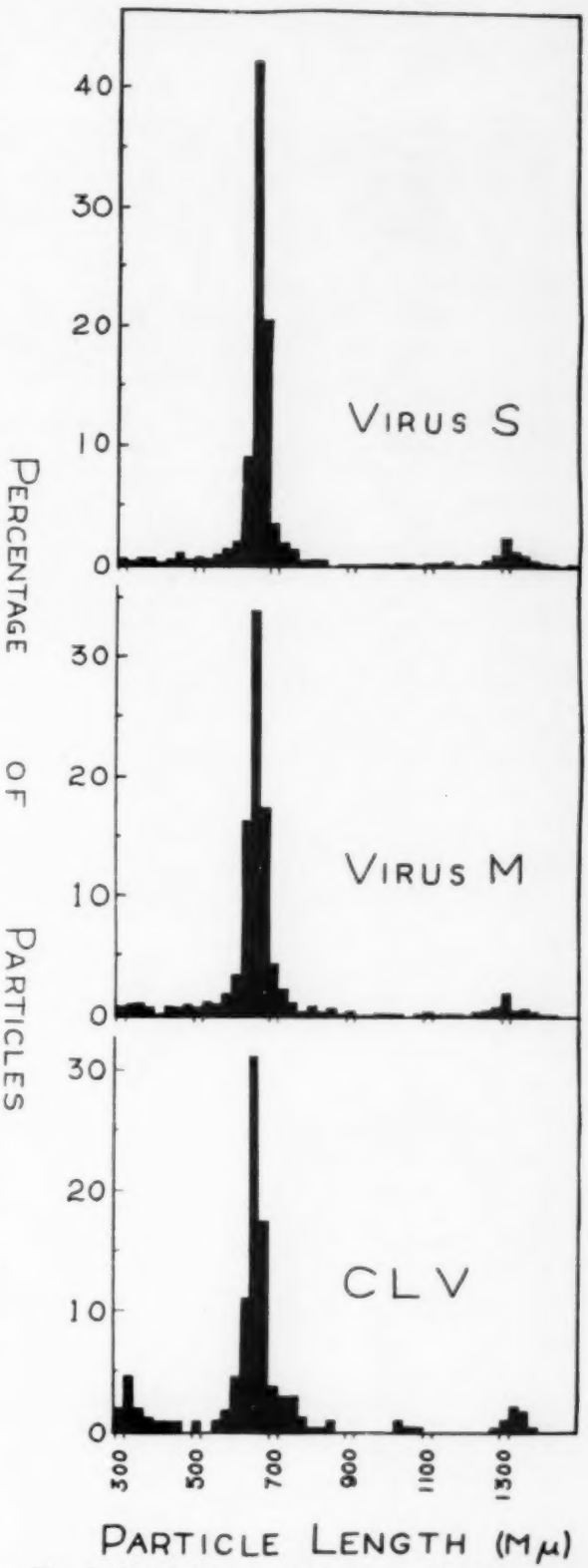

Fig. 2. Distribution of particle lengths of potato virus S (top); potato virus M (middle); and carnation latent virus (bottom).

TABLE 1.—*Normal and double particle lengths of virus S, virus M, and CLV*

Virus	Source	Host plant	Number of Particles	Normal length (mμ)	% within curve	Double-length (mμ)	% within curve
S	Arran Victory	Potato	199	656	71		
	Erdgold	Potato	407	658	81		
	Industrie	Potato	143	656	64		
	Sdlg. 41956	Potato	191	649	71		
			940	657	82	1310	5
M	LRM	*S. demissum*	207	649	81		
	IVM	*Tomato, D. metel*	143	651	79		
	Paracrinkle	*D. metel*	220	656	79		
	D1102	*Tomato, G. globosa*	555	651	76		
			1125	651	80	1299	6
CL	Carnation	Sw. William	234	652	68	1328	5

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THE EFFECT OF LOCAL INFECTION WITH TOBACCO MOSAIC VIRUS ON RESPIRATION IN LEAVES OF NICOTIANA GLUTINOSA[1]

Akira Yamaguchi and Tokuzo Hirai[2]

SUMMARY

The respiration of half-leaves of *Nicotiana glutinosa* inoculated with TMV was higher than that of uninoculated halves after local necrotic lesions appeared. This increased ratio of respiration seemed to vary considerably with the various stages of development of the lesions. Increase of virus was rapid in the inoculated leaves of *N. glutinosa* before the appearance of lesions. The respiratory rate did not increase until the lesions appeared. It was tentatively concluded that the increased rate of respiration is not associated with the rapid increase of virus, but rather reflects the formation of local necrotic lesions.

Many publications compare the rates of respiration of healthy and virus-infected plant tissues, with divergent and apparently contradictory results (1). However, many factors may govern the results. For instance, Owen (4), using tobacco leaves infected with TMV (tobacco mosaic virus), showed that respiratory rate depends on (a) the interval after inoculation; (b) the physiological state of the plants; (c) environmental conditions during growth; (d) the leaves chosen; (e) the mode of expressing the results.

By floating detached leaves on a nutrient solution, Takahashi (9) obtained a physiologically uniform state of leaf, necessary for the study of leaf respiration. He said that the respiratory rate of virus-infected half-leaves tends to be slightly lower than that of uninoculated controls. On the other hand, the authors, using the same leaf-culture method, could detect no significant differences between TMV-inoculated and uninoculated tobacco half-leaves until 4 days after inoculation; however, the bean leaf (*Phaseolus vulgaris* L. var. Ohtenashi) inoculated with the same virus, which produced local necrotic lesions, showed a definite respiratory increase over uninoculated leaves (10).

The findings reported herein indicate that respiration in *Nicotiana glutinosa* leaf with necrotic lesions increases in the same manner as does the bean leaf. The present report examines these facts, together with the problem of whether the change is due to lesion development or virus multiplication within the inoculated leaf.

MATERIALS AND METHODS.—*N. glutinosa* was grown in a greenhouse in 4-in. pots for about 10 weeks and topped before inoculation. The plants were trimmed so that only 4–6 large leaves remained, at the middle of one stem. The upper surface of all the leaves was uniformly rubbed with carborundum suspended in distilled water. After being washed in a stream of tap water, each half-leaf was inoculated by rubbing with TMV (ordinary strain found in Japan) partially purified from systemically infected tobacco leaves, using Schneider's chloroform emulsion method (8). The other half-leaf served as control. These plants were kept in a constant-temperature chamber under fluorescent light for about a 10-hour day.

Measurements of oxygen consumption were made with a Warburg respirometer. At various intervals after inoculation, described later, 30 disks, 5 mm in diameter, were punched out of the inoculated and uninoculated half-leaves. These disks were floated on 2 ml of 0.1*M* acetate buffer (pH 5.0) in a flask. All measurements were recorded for 60 minutes in the dark at 30°C, after 30 minutes of equilibration to eliminate the effect of injury by wounding. At the end of the experiments, the disks were dried and weighed, and the respiratory rate per gram of dry matter (Q_{O_2}) was calculated.

The actual increase of TMV from inoculation within the *N. glutinosa* leaf was determined by the routine method of inoculating *N. glutinosa* and of counting the numbers of local lesions produced.

RESULTS.—In a preliminary experiment it was found that the opposite halves of a healthy leaf of *N. glutinosa* remained relatively uniform in rate of respiration, and that at 25°C there was no detectable virus in the uninoculated half-leaf until 5 days after inoculation of the opposite half-leaf. It was therefore possible to designate the inoculated and uninoculated half-leaves as infected (I) and healthy (H), respectively.

Respiration.—The respiratory rates of the opposite inoculated and uninoculated half-leaves were determined simultaneously, with duplicate manometers for each experiment. Fig. 1 shows the ratio of respiration at 25°C of inoculated to uninoculated half-leaves at the different times after inoculation. Under the conditions outlined above, local necrotic lesions became visible about 36 hours after inoculation. The ratio of respiration of inoculated to uninoculated tissues was about 1.0 or lower within 30 hours of inoculation, when there was no appearance of local lesions; however, after 36 hours the ratio increased gradually, in proportion to lesion enlargement, and on the 3rd day after inoculation this value finally reached 1.2–1.4. Then, on the 4th day, it declined again, to nearly 1.0. On the other hand, local necrotic lesions at 20°C became visible about 40 hours after inoculation, and

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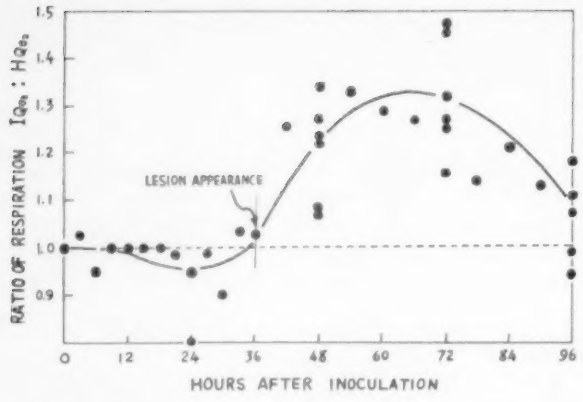

Fig. 1. The ratio of respiration of *N. glutinosa* half-leaves inoculated with TMV at 25°C to opposite half-leaves not inoculated. Each symbol indicates values obtained in independent but comparable experiments.

their enlargement was slower than those that developed at 25°C. The respiratory ratio in this case (I:H) increased gradually from the time of lesion appearance until 4 days after inoculation (Fig. 2-B).

Respiratory quotient.—The respiratory quotient (R.Q.) is assumed to reflect the nature of the metabolites oxidized during respiration. If the respiratory increase of the half-leaf on which local lesions develop is caused by utilization of unusual substrates produced in the inoculated leaf, the R.Q. values would be expected to vary correspondingly. The R.Q. was measured by Warburg's direct method. A typical result of the experiments at 25°C is presented in Table 1. These values, for the 3rd and 4th day after inoculation, were nearly identical in both the inoculated and uninoculated half-leaves although the I:H ratio of respiration increased to 1.46:1.00 after the 3rd day.

Virus increase.—The upper surfaces of 16 leaves of 4 *N. glutinosa* plants at 20°C were inoculated uniformly with TMV. Immediately after, 30 disks (10 mm in diameter) were punched out of each leaf. These disks were ground, and the leaf extracts produced were clarified by centrifuging at 1000 rpm for use in an infectivity assay. At different intervals after inoculation, another 30 disks were obtained from the same leaves, and their extracts were prepared in like manner. The virus infectivity was assayed by comparing the number of local lesions produced on *N. glutinosa* half-leaves inoculated with the leaf extracts to those produced on the opposite halves inoculated with standard inoculum. At least 10 half-leaves were used for each experiment. The relative infectivity of the leaf extracts obtained successively after inoculation is shown in Fig. 2-A. The infectivity first decreased, and did not tend to increase until about 30 hours after inoculation. Then the virus began to increase at a rapid rate before the appearance of lesions; thereafter, the virus increase stopped—about 48 hours after inoculation.

Discussion.—Owen (3, 4) stated that TMV-infection increased the rate of respiration of tobacco leaves within an hour of inoculation; however, this increase was maintained at the same rate continuously for over 2 weeks and no evidence was presented of any change in respiration rate after the initiation of rapid increase of the virus. From these results, he suggested that the increase in rate of respiration was caused by a change in the infected cells that occurred before virus multiplication. In contrast, as shown in this report, the respiration of inoculated leaves of *N. glutinosa* increased after the local lesions became visible. The increased rate of respiration of the bean leaves inoculated with TMV has already been shown (10). Owen (6) recently demonstrated that the respiration rate of *N. glutinosa* leaves inoculated with TMV increased when local lesions appeared, and that the rate of the increase was related to the number of lesions. In contrast, it is evident in the present report that the respiratory ratio (I:H) varies with the developmental stages of lesions.

Table 1.—*Respiratory quotient (R.Q.) of uninoculated and TMV-inoculated half-leaves of N. glutinosa*

Days after inoculation	R.Q. Healthy	R.Q. Infected	IQ_{O_2}:HQ_{O_2}
3	0.95	0.95	1.46
	0.89	0.80	1.32
4	0.89	0.83	1.19
	0.98	0.96	0.98

In our experiments on virus increase, the infectivity of *N. glutinosa* at various intervals after inoculation

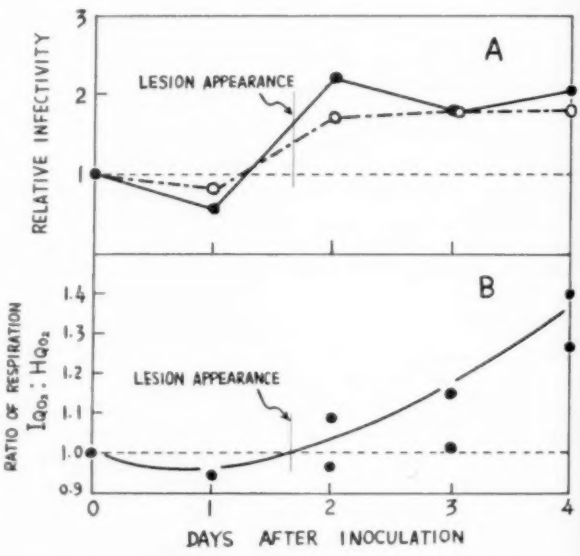

Fig. 2. Increase of virus within, and respiratory increase of, *N. glutinosa* leaves inoculated with TMV at 20°C. **A)** Relative infectivity of *N. glutinosa* leaves at various times after inoculation. Both solid and dotted lines show the results of independent but comparable experiments. **B)** The ratio of respiration of inoculated half-leaves to uninoculated half-leaves. Each symbol indicates values obtained in independent but comparable experiments.

with TMV decreases before it increases, and its increase stopped about 2 days after inoculation. This result is fairly consistent with Harrison's data (2). In spite of the fact that a rapid virus increase was confirmed before the appearance of lesions, the increase of the rate of respiration was not observed within this period of virus increase, but was observed during lesion development. These facts may imply that the respiratory increase is not related to the rapid virus increase, but reflects the development of local necrotic lesions. However, since, as has been shown by Rappaport and Wildman (7), these phenomena may not be so strictly separated, it could not be decided precisely what changes induced the rise in rate of respiration. According to Owen (5), who obtained a respiratory increase of tobacco leaf inoculated with tobacco etch virus after the appearance of external symptoms, a change of respiration due to an initial virus increase may occur; however, it was too small to be detectable, because the inoculum used was more diluted than TMV. Nevertheless, in our experiment, there was no rise in the rate of respiration in the *N. glutinosa* infected with TMV corresponding to this initial virus increase, but rather a decrease of respiration, usually within 24–30 hours of inoculation. From this phenomenon, it becomes impossible to find a logical interpretation until the picture is more complete.

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PHYTOPATHOLOGICAL NOTES

An Inhibitor of Verticillium albo-atrum in Cotton Seed. GARBER, R. H., AND BYRON R. HOUSTON.[1] In a study of the mode of penetration of cotton roots by *Verticillium albo-atrum* Reinke & Berth. it was very difficult to infect seedlings just emerging from the seed coat. A study was made to determine whether some factor in young seedlings was preventing growth of the fungus. Wilt-susceptible Delta-pine 15 and resistant Acala 4-42 cotton seeds were placed in warm potato-dextrose agar in Petri dishes, and a suspension of Verticillium spores was added when the agar was cool. The fungus developed everywhere on the agar except in the area immediately surrounding the seeds (Fig. 1). There was no evidence of changed pH in this area.

In similar tests, seed coats separated from embryos were separately placed on agar seeded with Verticillium spores. The fungus developed to some extent around the embryos, but not around the seed coats (Fig. 1).

The unidentified inhibitor extracted from 25 g of seed in 30 ml of hot water (160°F for 1 min.) was sufficiently concentrated to inhibit development of Verticillium in seeded agar plates. After extraction with hot water the cotton seeds were placed on agar seeded with spores of Verticillium, and such seeds failed to demonstrate any inhibitory zone surrounding them. Tests were made to determine whether the inhibitor in the seed coat was a metabolic product of the embryo that would reaccumulate after its removal by hot water. Seeds were stored at room temperature for 1 month after extraction of the inhibitor from the seed coat. Tests made after the storage period failed to show any evidence of reaccumulation of the inhibitor.

Petri plates of potato-dextrose agar containing Acala 4-42 cotton seeds were seeded with Verticillium spores as described previously. These plates were observed microscopically 16 hours after seeding. Germination of conidia was delayed in definite, almost circular, areas surrounding each seed. However, when ungerminated conidia were removed from the areas of inhibition along with 1-mm agar squares and placed on sterile agar slants, they produced abundant growth.

[1] Contribution from the Crops Research Division, ARS, U. S. D. A., and Agricultural Experiment Station, University of California, Davis.

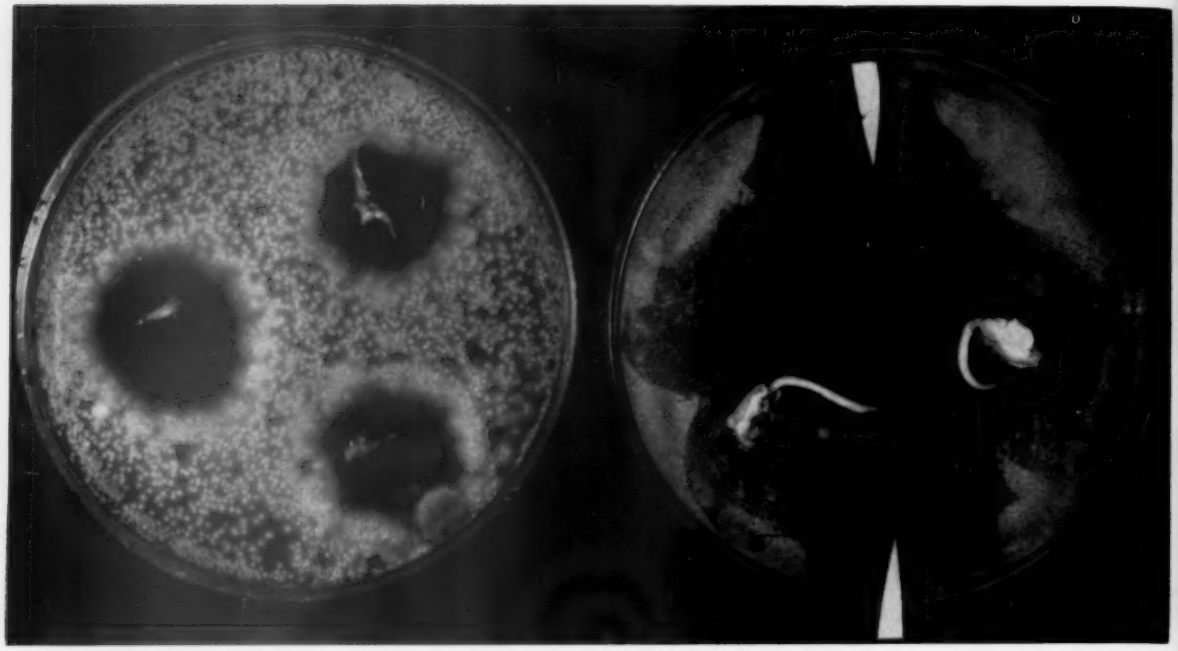

Fig. 1. Left: Three Delta-pine 15 cotton seeds germinated on potato-dextrose agar seeded with *Verticillium albo-atrum*. The inhibition zone surrounds each seed. **Right:** Seed coats and embryos separated after partial germination and prior to being plated on agar seeded with Verticillium spores. The larger and more definite zones of inhibition surround the seed coats (arrows).

This indicates that, at least at this early stage of sampling, the inhibitory substance is fungistatic, not fungicidal. Conidia left in the areas around the seeds had not germinated 2 weeks after seeding.

Further tests are in progress to determine the nature of the substance and its possible relation to resistance to Verticillium wilt and other diseases in cotton.—Department of Plant Pathology, University of California, Davis, California.

Growth of Six Races of Phytophthora infestans on Artificial Media.[1] H. David Thurston, Peter Wilde, and Theodore W. Sudia.[2] French (3), working with races 0 and 4, and Hodgson (4), working with races 0; 1; 1,2; and 1,2,3, of *Phytophthora infestans* (Mont.) d By., both found that these physiologic races differed in their ability to utilize various nitrogen and carbon sources. Similar studies made at Minnesota with 6 physiologic races (0; 1; 4; 1,4; 2,4; and 1,2,4) indicated similar differences, but tests with several isolates of the same race suggested that these differences might not be characteristic of the races as determined by pathogenicity on differential potato clones. Hodgson (4) also suggested "that a study of the nutrition of a number of isolates of the same race should be made before the differences that have been demonstrated between single isolates are accepted as being characteristic of the races concerned." The experiment reported herein was made with this suggestion in mind.

Isolates were cultured on Lima-bean agar medium made according to Thurston (6) and a medium developed at Minnesota (hereinafter called the synthetic medium): KH_2PO_4, 0.5 g per liter; $MgSO_4 \cdot 7H_2O$, 0.25 g per liter; thiamine, 1.0 ppm; dextrose, 25.0 g per liter; DL-aspartic acid, 2.38 g per liter; rotted and washed agar (after Winogradsky (5)), 15.0 g per liter.

Eight Petri plates of each medium were each inoculated in the center with 5-mm disks cut from the margin of a vigorously growing colony of each isolate. The inoculated plates were incubated at 20°C. The physiologic races studied (Table 1) were identified by their pathogenicity on differential clones according to the system of Black et al (1). The 6 isolates of race 0 had all been isolated from the same potato field. The mean diameters after 7 days of 8 colonies of each isolate are given in Table 1. F-ratios are shown as determined by analysis of variance, together with groups of isolate means not significantly different as determined by the Student-Newman Keuls multiple range test (2).

The analysis showed that in no case is there a significant difference between replications, indicating low variability due to technique. However, the differences among races depend in part on the medium

[1] Paper No. 4042, Scientific Journal Series, Institute of Agriculture, University of Minnesota, St. Paul. Research supported by United States Atomic Energy Commission, Contract No. AT(11-1)-42.

[2] Respectively, formerly Research Fellow, now with Rockefeller Foundation, Bogota, Colombia; formerly Research Assistant, now with Institut für Pflanzenpathol., Göttingen, Germany; and Research Fellow, University of Minnesota.

TABLE 1.—*Mean diameter (mm) of 8 colonies each of 6 isolates of race 0 and of races 1; 4; 1,4; 2,4; and 1,2,4 on synthetic medium and Lima-bean agar medium after 7 days, including F values*

Synthetic medium						Lima-bean agar					
Isolate	Race	Diam.	.05	.01[a]	F value	Isolate	Race	Diam.	.05	.01[a]	F value
510	0	25.6	]	]		507	0	65.3	]	]	
522	0	23.8	]	]		513	0	61.8	]	]	
500	0	22.9	]	]	48.50**	500	0	61.4	]	]	7.22**
513	0	16.8	]	]		503	0	58.8	]	]	
503	0	15.6	]	]		522	0	56.1	]	]	
507	0	5.0	]	]		510	0	44.8	]	]	
469	1	19.8	]	]		469	1	76.9	]	]	
528	1,4	18.8	]	]		493	2,4	72.0	]	]	
470	4	16.6	]	]	3.10*	470	4	62.1	]	]	65.62**
493	2,4	15.5	]	]		528	1,4	54.6	]	]	
498	1,2,4	15.1	]	]		498	1,2,4	54.4	]	]	

[a] Means enclosed by brackets do not differ significantly from one another at indicated levels of significance.
* Significance at 5% level.
** Significance at 1% level.

in which they grow. For example, of 6 isolates of race 0, isolate 507 grew fastest on Lima-bean agar and slowest on the synthetic medium; isolate 510 did the opposite.

When grown on the synthetic medium, the 6 isolates of race 0 showed far greater differences than the differences among the other 5 races studied. The means of the other 5 races (Table 1) fall into 2 groups at the 5% level of significance, and into only one group at the 1% level. This is also indicated by the F-ratios, which are significant at the 5% level but not at the 1% level. On the other hand, when grown on Lima-bean agar the 6 isolates of race 0 differ but little in colony size, isolate 510 being the only one significantly different from the rest. Large differences occur among the 5 other races on this medium, each of which differs from the rest at the 5% level.

It is concluded that many isolates of a given race should be studied before differences in ability to utilize specific compounds can be accepted as racial characters, and that many of these characters are probably inherited independently of factors for specific pathogenicity.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

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Inhibition of Starch Formation by Claviceps purpurea.[1] W. P. CAMPBELL.[2] Although there has been considerable discussion as to the source of honeydew secreted from the florets of plants infected by *Claviceps purpurea* (Fr.) Tul., most workers have considered that the sugary phase of this exudate is produced by the host under stimulation of the parasite.[3, 4, 5] Others,[6, 7] in opposition to this view, considered it a product of the fungus. Fuchs and Pöhm[4] demonstrated that honeydew contained glucose, fructose, sucrose, and 4 unidentified sugars. Glucose and fructose were dominant, and at least 2 of the other 4 were identical to sugars found in the nectar of normal plants. Maltose was not present. Taber and Vining[8] found that *C. purpurea* could not utilize starch as a carbon source, and that maltose was utilized only slightly.

The cereal seed normally stores starch, and it is the organ that is parasitized by the ergot fungus. A sclerotium replaces the seed, and this body contains no starch. It may be assumed that no starch is formed or that it is all used by the fungus. The results reported herein indicate that the first assumption is the valid one.

[1] Contribution No. 1728 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] Associate Plant Pathologist.
[3] Engelke, C. 1902. Neue Beobachtungen über die Vegetations-Formen des Mutterkornpilzes *Claviceps purpurea* Tulasne. Hedwigia 41: 221-222.
[4] Fuchs, L., and M. Pöhm. 1953. Zur Kenntnis des Honigtaues. Sci. Pharm. 21: 239-241.
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[7] Schweizer, G. 1941. Über die Kultur von *Claviceps purpurea* (Tul.) auf kaltsterlisierten Nährböden. Phytopathol. Z. 13: 317-350.
[8] Taber, W. A., and L. C. Vining. 1957. A nutritional study of three strains of *Claviceps purpurea* (Fr.) Tul.

A starch-free potato extract obtained by grinding tubers and filtering the expressed sap through powdered talc was added to a solution of the potassium salt of glucose-1-phosphate, following the method of Hanes.[9] After digestion at room temperature for 10 minutes, the addition of iodine in potassium iodide indicated that starch was present. When a small amount of filtered honeydew or aqueous extract of powdered ergot was added to the tubes at the same time as the potato extract, no starch could be detected by the iodine test. In a check test it was found that ergot extract did not affect the formation of the starch-iodine complex. If, however, the ergot extract or honeydew was boiled before it was introduced, there was no inhibition of starch synthesis.

These results indicate that *C. purpurea* possesses a starch phosphorylase inhibitor that prevents the conversion of sugars to starch and preserves the sugars of the host in forms available to the fungus.—Plant Pathology Laboratory, Science Service, Edmonton, Alberta.

Failure of Thrips to Transmit an Isolate of Tomato Spotted Wilt Virus.[1] JOHN P. JONES.[2] For a number of years a virus disease of tomatoes, the symptoms of which resemble those described for tomato spotted wilt virus, has been observed in the Lincoln area. The disease shows considerable variation from year to year, and has occurred epiphytotically.

An attempt to identify the virus was made by comparing the Lincoln virus with an isolate of tomato spotted wilt virus obtained from Dr. P. G. Smith, of the University of California. Symptom production of the two isolates was compared over 15 months on tomato (*Lycopersicum esculentum* L.), tobacco (*Nicotiana tabacum* L.), petunia (*Petunia hybrida* Hort.), nasturtium (*Tropaeolum majus* L.), jimson weed (*Datura stramonium* L.), and *Nicotiana glutinosa* L. No symptom differences were observed.

Parallel tests, using expressed juice from infected tobacco plants, were carried out on the longevity, thermal inactivation point, and dilution end-point of the two isolates. The longevity of both viruses in 1-ml lots of juice at room temperatures was 1–2 hours. With sodium sulfite buffer solution[3] added to the juice, the longevity of the Lincoln isolate was increased to 3–4 hours, and that of the California isolate to more than 5 hours. The thermal inactivation point of both viruses was 42–44°C in both buffered and unbuffered juice. The dilution end-point of the Lincoln isolate was 1:10–1:100, and that of the California isolate was greater than 1:500. It was observed throughout these tests that the Lincoln isolate was much more labile *in vitro* than was the California isolate.

Both viruses were transmitted from nasturtium to nasturtium by the dodder *Cuscuta campestris* Yuncker.

Several attempts were made to transmit the Lincoln virus by means of *Thrips tabaci* Lindemann. The culture of thrips was identified by Dr. S. F. Bailey, of the University of California.

The first attempt at transmission was carried out with the technique described by Norris.[3] Thrips larvae were placed on caged nasturtiums infected with the Lincoln virus. At 5 days, 10 healthy young nasturtiums were placed in the cage near the infected plants. Eleven days later the 10 plants were removed and fumigated in a fumagatorium with a tobacco-derivative fumigant. All 10 plants showed numerous and extensive feeding marks on the leaves. No infection developed.

Owing to the lack of transmission in the first test, a second, more closely controlled, attempt was made. A mixed culture of adult and larval thrips was placed on caged Lincoln-virus-infected nasturtiums. Daily observation showed that all the adult thrips disappeared after 1 week, and no adults appeared until 11 days thereafter. It was believed that the adult thrips disappeared because they died, and that those appearing later had emerged from larval pupation. Starting 20 days after the thrips were colonized on the infected nasturtiums, individual adult thrips were transferred to single insect cages on healthy young nasturtium and tomato plants, 1 per plant. A thrips was kept on a test plant until feeding marks were distinct, when the plant was fumigated and held for observation. Transfer of the thrips to test plants continued over 2 months and involved 53 nasturtium and 24 tomato test plants. No virus infection occurred.

A third test slightly modified the second. Only larval thrips were used to colonize infected nasturtiums, and the resulting adults were individually transferred to test nasturtiums. Individual transfers, started 9 days after colonization, continued for 5 days to a total of 20 test nasturtiums. As in the previous tests, no infection resulted.

Since previous evidence showed the Lincoln virus to be TSW virus or a strain thereof, the lack of transmission by *T. tabaci* was puzzling, particularly since the peculiar conditions of larval acquisition and incubation that are requisite for thrips transmission[4] were apparently fulfilled. Added to this is the fact that Norris[3] obtained transmission of TSW virus by using *T. tabaci* and nasturtium. One possible explanation for the lack of transmission is that the virus titer of

[1] Published with the approval of the Director as paper No. 887, Journal Series, Nebraska Agricultural Experiment Station. This work is part of a study conducted in partial fulfillment of the requirements for the M.S. degree.

[2] Formerly Research Assistant, Department of Plant Pathology, University of Nebraska. Presently Plant Pathologist, Agr. Research Service, U. S. Department of Agr., Stoneville, Mississippi.

[3] Norris, D. O. 1946. The strain complex and symptom variability of tomato spotted wilt virus. Council for Sci. and Ind. Res. Australia Bull. 202. p. 1-51.

[9] Hanes, C. S. 1940. The breakdown and synthesis of starch by an enzyme system from pea seeds. Proc. Roy. Soc. (London) B. 128: 421-450.

[4] Bald, J. G., and G. Samuel. 1931. Investigations on "spotted wilt" of tomatoes. II. Council for Sci. and Ind. Res. Australia Bull. 54: 1-24.

this particular isolate in the host plant is too low for insect acquisition. This hypothesis is strengthened by the results of the dilution and aging tests described.

Unfortunately, thrips transmission tests were not attempted with the California isolate of TSW. There have been a number of reports of successful transmission of TSW virus with *T. tabaci*.[5] Conversely, some negative or doubtful results have also been obtained. Samuel, Bald, and Pittman were unable to obtain transmission of TSW virus with *T. tabaci* in one study,[6] but later reported transmission in another.[7] They noted, however, that the thrips *Frankliniella insularis* Franklin was a much more efficient vector than *T. tabaci*. Smith, on the other hand, reported transmission of TSW virus in an early study,[8] then failure in later trials (oral communication). It thus seems that the relationship between *T. tabaci* and the TSW virus complex may have some unexplained aspects that might be elucidated by further study.—Department of Plant Pathology, University of Nebraska, Lincoln.

The Early, Dwarf Symptom of Bacterial Ring Rot of Potato in Idaho. JAMES W. GUTHRIE.[1] The common symptoms of bacterial ring rot of potatoes have been quite accurately described by a number of workers. An early-season symptom occurring on Russet Burbank and Early Gem varieties in Idaho, however, has not been described. Seed pieces of these two varieties, when infected with *Corynebacterium sepedonicum* (Spiek & Kott) Skapt. & Burkh., produce plants that show these symptoms in about 45 days.

Stems originating from infected eyes produce a plant that is dwarfed by reduced internodal growth. The leaves and leaflets at the top of an infected stem are contorted and slightly twisted (Fig. 1). The plant becomes darker green and the leaflets thinner than normal. The leaves of the plants have a velvety texture. Necrotic margins frequently develop on the leaflets. Shortly thereafter the plants begin to wilt rapidly and all affected stems become progressively necrotic and subsequently die. If some eyes are not infected, symptoms are restricted to the stems and foliage from the infected eyes. It is thus possible for only one or two stems per hill to exhibit the dwarfed or rosette growth habit.

The symptom expression of yellow dwarf in Wisconsin[2] and that of the early, dwarf symptom of bacterial ring rot of potato in Idaho are somewhat similar. Certain characteristics are dissimilar, such as the lack of rugosity of apical leaves. Likewise, pith necrosis of stems is not usually associated with ring rot unless secondary soil organisms are contributing causes to this condition. Infected seed pieces from emerging hills are not hard and glossy in texture but frequently are rotted or at least the vascular ring will show some degeneration. At the present time yellow dwarf of potato has not been found in southeastern Idaho. None of the other virus diseases of potato commonly found in Idaho such as seed-borne leafroll, crinkle mosaic, rugose mosaic, or witches broom express symptoms that might be confused with the early ring rot symptoms. Potato black leg may cause some confusion particularly if only a single stem of a hill is dead. On examination of the underground stem, the presence of the blackened tissue will normally be sufficient to separate black leg from ring rot. With ring rot, particularly prior to death of the stem, the presence of a creamy-yellow ooze exuding from the vascular system of the stem severed near the junction of the stem and seed-piece further aids in the diagnosis. The laboratory gram stain can also assist in diagnosis.

The above description of the dwarf-rosette symptom of bacterial ring rot of potato was first observed under field conditions. Confirmation of the relation between the causal bacterium and observed symptoms by employing Koch's postulates was achieved through greenhouse tests during the winter of 1956-57, and field tests in 1957 and 1958. Fig. 1 shows a potato plant whose seed piece was inoculated by jabbing the eyes

Fig. 1. Dwarf-rosette expression of potato ring rot on Early Gem variety.

[1] Assistant Plant Pathologist, Department of Plant Pathology, University of Idaho, Aberdeen Branch Experiment Station, Aberdeen, Idaho.

[2] Walker, J. C., and R. H. Larson. 1939. Yellow dwarf of potato in Wisconsin. J. Agr. Research 59: 259-280.

[5] Sakimura, K. 1940. Evidence for the identity of the yellow-spot virus with the spotted-wilt virus: Experiments with the vector *Thrips tabaci*. Phytopathology 30: 281-299.

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Fig. 2. Quick green-wilt expression of potato ring rot on Bliss Triumph variety.

with a sterile needle dipped in a culture of *C. sepedonicum* and planted in the field on May 15 and photographed on June 30.

Later in the season the stems of previously symptomless infected plants develop a fast wilt commonly associated with this disease. Frequently the tubers on such plants are rotted or partially rotted (Fig. 2). This does not occur with early-dwarf plants since they normally succumb before tubers are formed.—Plant Pathology, Aberdeen Branch, Idaho Agricultural Experiment Station, Aberdeen.

The Mineral Oil Seal Method for Maintaining Cultures of Tilletia caries for Long Periods. E. L. KENDRICK.[1] Research on the physiologic specialization and genetics of *Tilletia caries* (DC.) Tul. requires that large numbers of monosporidial lines be isolated and maintained in culture, and many of them retained indefinitely. Monosporidial lines of *T. caries* grow well on a PSA medium (potato-sucrose-agar, 200: 10:20 g), but in normal stock-culture storage must be transferred at least once every 6 months to ensure continued viability. Longer storage without transfers would be desirable, especially for the many lines that are brought back into use only after storage for a year or more. Although storage under a mineral-oil seal was found successful in holding cultures of many different fungi for long periods without transfer,[2] species of *Tilletia* were not among those tested. A study was undertaken to determine whether cultures of *T. caries* could be maintained for long periods by this method.

Cultures of race T-9 of T. caries[3] were used—2 sets: 11 and 10 monosporidial lines, respectively from spores 282 and 283. Following the method of Buell and Weston,[2] 3 cultures of each line, growing actively on PSA slants, were covered with sterile mineral oil to a depth of 1 cm above the top of the slant. These were stored upright under refrigeration at 10°C throughout the period of study. A comparable set of these same lines was maintained without the oil seal. Transfers from 1 culture of each line were made to fresh PSA slants at 6-month intervals for 3½ years. Usually, 3 successive transfers to fresh PSA slants were necessary before an oil-stored culture resumed a rate of growth comparable with that of its counterpart stored without the oil seal and transferred every 6 months. After 3½ years under oil, all 21 monosporidial lines retained their viability, though some lines showed a noticeable change in growth type.

Sporidial growth type in monsporidial lines is essential to successful study of genetic phenomena in *Tilletia* spp. Newly isolated lines apparently are inherently either mycelial or sporidial. The former is usually stable for that growth type, but some of the latter may change from sporidial to mycelial growth after several transfers in culture. In the course of normal culturing, many of the monosporidial lines of spore 282 shifted to mycelial growth, whereas the lines of 283 remained quite stable for sporidial growth.

After 2 years of storage under oil, all but 1 line of spore 283 exhibited sporidial growth comparable with that of the stock cultures, whereas several lines from spore 282 produced mycelial growth, in contrast to their original sporidial growth. Other cultures of these same lines of spore 282, however, exhibited sporidial growth when transferred from cultures stored 2½ years under oil. After 2½ years under oil, several of the lines from spore 283 apparently shifted to a mycelial growth; but other replications of these same lines exhibited sporidial growth when transferred from cultures stored under oil for 3 years. After 3½ years under oil, cultures of all the lines of spore 282 produced mycelial-type growth, while cultures of 4 of the 10 lines of spore 283 had shifted to a mycelial growth.

Sex compatibility relationships did not change in the monosporidial lines stored under oil for 3½ years. Infectivity was retained as shown by the fact that compatible pairs of lines held in oil for 2 years produced infection on the susceptible spring wheat Red Bobs.

The results indicate that monosporidial isolates or lines of *T. caries* can be maintained successfully under a mineral oil seal for at least 3½ years. However, if a sporidial type of growth is to be maintained in a monosporidial line, several replications should be kept under oil, and transfers should be made at least once every 2 years.

[1]Pathologist, Crops Research Division, ARS, U. S. D. A., Pullman, Washington.

[2]Buell, Caroline B., and W. H. Weston. 1947. Application of the mineral oil conservation method to maintaining collections of fungous cultures. Am. J. Botany 34: 555-561.

[3]Rodenhiser, H. A., and C. S. Holton. 1937. Physiologic races of *Tilletia tritici* and *T. levis*. J. Agr. Research 55: 483-496.

SINGLE-SPORE INOCULATION AND INCREASE APPLICABLE TO THE RUST FUNGI[1]

R. D. Milholland and H. C. Young, Jr.

Once a rust race becomes contaminated or mixed with another race, purification is difficult, for there are no morphological differences between the urediospores of different races. Common practice in purifying races is to increase a culture from a single pustule, which usually involves thousands of urediospores. Two pustules produced so closely together that they appear as one pustule, or foreign spores lodged in a pustule, complicate the problem of obtaining pure cultures in this manner. Inoculation of the leaves of living plants with single rust spores has been accomplished, but the contamination problem is formidable. To overcome these difficulties, the following technique for a single-spore inoculation has been developed and used successfully in purifying races of *Puccinia recondita* Rob. ex Desm.

Plants of a susceptible wheat variety were grown in 4-in. pots in a room isolated from all rust sources for 10 days preceding inoculation. The primary leaves to be inoculated were rubbed between moistened fingers to reduce their surface tension, sprayed with distilled water, and then excised and placed in a divided Petri dish containing a solution of benzimidazole (40 ppm). The divided-type Petri dish was used to prevent the leaf from becoming submerged in the solution. Detached wheat leaves placed in 40 ppm benzimidazole solution will retain their normal reaction to rust,[2] which they may not do in nutrient solutions or in plain water.[3]

Fresh inoculum from a 10-day-old rust pustule was used. Spores from a single pustule were removed with a spatula-type needle and placed in a drop of water on a glass slide, which was allowed to dry. Single spores were easier to pick up if the drop of water was allowed to dry first. The Petri dish containing the excised leaf was then placed on the stage of one binocular dissecting microscope, and the glass slide on the stage of a second microscope for ease in handling. With a sterilized glass needle, a single urediospore was removed from the glass slide and placed in a minute drop of water on the excised leaf.

After inoculation, the Petri dish containing the excised leaf was incubated 12 days at 18–20°C under a light intensity of about 1500 ft-c for 12 hours daily.

Urediospores from the pustule produced (Fig. 1) were increased on a susceptible wheat variety isolated from other sources of rust. The inoculum thus produced was brushed on a set of differential varieties for identification.

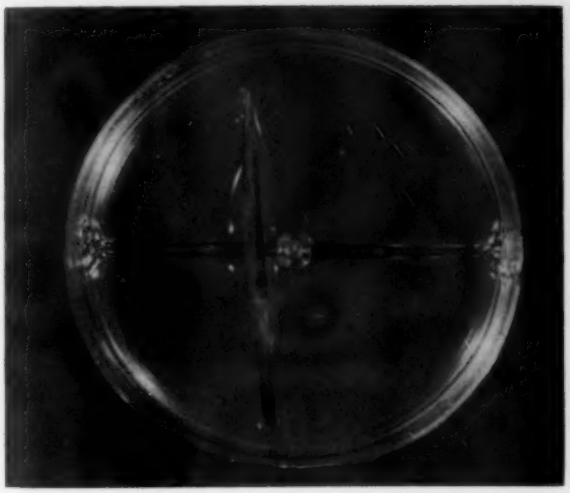

Fig. 1. A pustule of leaf rust produced from a single urediospore on a detached wheat leaf floated on a 40-ppm solution of benzimidazole 12 days after inoculation.

This method will also be of value in studying the effect produced by two urediospores of different races or different rusts germinating in close proximity.—Department of Botany and Plant Pathology, Oklahoma State University, Stillwater.

THE RELATIONSHIP OF CULTURE SUBSTRATE TO STAINING OF PHYTOPHTHORA SPECIES WITH ZINC-CHLOR-IODIDE

J. W. Bushong and J. W. Gerdemann[1]

Kaufmann and Gerdemann[2] reported differences in the staining of 6 species of *Phytophthora* with zinc-chlor-iodide,[3,4] and suggested that this test might be of some value in distinguishing species. They reported that mycelia of *P. infestans* (Mont.) de Bary, and *P. erythroseptica* Pethyb., and 7 isolates of *P. sojae* Kaufm. & Gerd. stained a deep bluish-red, whereas *P. megasperma* Dreschs., *P. cryptogea* Pethyb. & Laff., and 3 isolates of *P. cactorum* (Leb. & Cohn.) Schroet. stained faintly. The medium on which the isolates were grown was not reported.

Further investigation has revealed that the culture media on which the organism is grown may affect the intensity of the stain. The same 6 species of *Phytophthora* were grown on hemp seed in water (3 hemp seeds autoclaved in 12 ml of water) and Difco cornmeal agar. Three isolates of *P. sojae*, and 1 isolate each of *P. infestans* and *P. erythroseptica*, stained darkly when grown on either media. An isolate of *P. megasperma* that stained faintly when grown on

[1] Ms. No. 475—Journal Manuscript Series, Oklahoma State University.

[2] Samborski, D. J., F. R. Forsyth, and C. Person. 1958. Metabolic changes in detached wheat leaves floated on benzimidazole and the effect of these changes on rust reaction. Can. J. Botany. 36: 591-601.

[3] Browning, J. A. 1954. Breakdown of rust resistance in detached leaves of normally resistant oat varieties. (Abstr.) Phytopathology 44: 483.

[1] Respectively, Research Assistant and Associate Professor of Plant Pathology.

[2] Kaufmann, M. J., and J. W. Gerdemann. 1958. Root and stem rot of soybean caused by *Phytophthora sojae* n. sp. Phytopathology 48: 201-208.

[3] Rawlins, T. E., and William N. Takahashi. 1952. Technics of plant histochemistry and virology. The National Press, Millbrae, California. 125 p.

[4] Zinc-chlor-iodide is commonly used as a reagent to test for the presence of cellulose.

hemp seed in water stained darkly when grown on corn-meal agar. One isolate of *P. cactorum* gave a weak reaction when grown on either medium. However, another isolate of *P. cactorum* that stained weakly when grown on hemp seed in water gave an intermediate reaction when grown on corn-meal agar, and a third isolate of *P. cactorum* that stained weakly when grown on hemp seed in water stained darkly when grown on corn-meal agar. An isolate of *P. cryptogea* that stained weakly when grown on hemp seed in water gave an intermediate to strong reaction when grown on corn-meal agar.

Differences in staining reaction could be due either to differences in the amount of cellulose, or to the presence of other compounds that may obscure the test for cellulose. The fact that isolates of the same species vary in staining reaction when grown on different media suggests that this test may be of little value in distinguishing species.—Department of Plant Pathology, University of Illinois, Urbana.

BOOK LISTINGS

ELLIOTT, FRED C. 1958. **Plant breeding and cytogenetics.** McGraw-Hill Co., New York. 395 p. $8.50

This book emphasizes the biological principles underlying the theory and methodology of plant breeding. It starts with an analysis of the botanical and biological nature of crop plants and follows with a discussion of the significant features of reproduction and the manipulation of variability. Among topics discussed are: methods of breeding, testing, and maintaining new varieties; the role of mutations and polyploidy in breeding; and a stimulating chapter on "Breeding for disease resistance." This chapter is especially recommended to plant pathologists since it presents several new concepts of disease control through host resistance. Material of a speculatory nature is also presented in the text to stimulate interest in new approaches. The book is neither a manual nor a cookbook but rather a well-integrated treatise on the application of cytogenetics to plant breeding.

MEVIUS, WALTER. 1958. **Taschenbuch der Botanik.** Erster Teil. Morphologie, Anatomie, Fortpflanzung, Entwicklungsgeschichte, Physiologie. [Pocketbook of Botany. Part one. Morphology, Anatomy, Reproduction, Development, Physiology.] Georg Thieme Verlag, Stuttgart. Ed. 17. 291 p. Paperback. Price $3.05.

A short, elementary text in botany, originally developed by Hugo Miehe, but now in its 17th revision. Thirty-eight pages are devoted to the morphology of vegetative plant bodies, 82 to anatomy, 14 to reproduction, 8 to development of seed plants, and 133 to physiology. The parts on morphology and reproduction include brief surveys of nonvascular plants. The book has 365 illustrations, mostly drawings. Many of the drawings are copies from familiar classics, but a number of new ones are introduced in the latest edition. The printing occupies 2/3 of each page, so that a wide margin is available for notes. There are 13 pages of subject index. No bibliographic references are given.

MILLS, HARLOW B. ET AL. 1958. **A Century of Biological Research.** Illinois Natural History Survey Bull. 27: 85-234. Free for a limited time from the Illinois Natural History Survey, Natural Resources Building, Urbana, Illinois.

This issue of the bulletin commemorates the centennial of the Illinois Natural History Survey. The first 14 pages describe the gradual evolution of the present institution from its beginning as the Museum of the Natural History Society of Illinois. Next are accounts of the research activities of the seven Sections. Although the Section of Botany was not established until 1921, botanical surveys were original activities of the institution. In 1870, Thomas J. Burrill began a series of reports in plant diseases and, in 1881, published his account of the fire blight of pome fruits. Surveys and research on plant diseases were expanded in 1921, and in 1931 the Section of Botany became the Section of Applied Botany and Plant Pathology.